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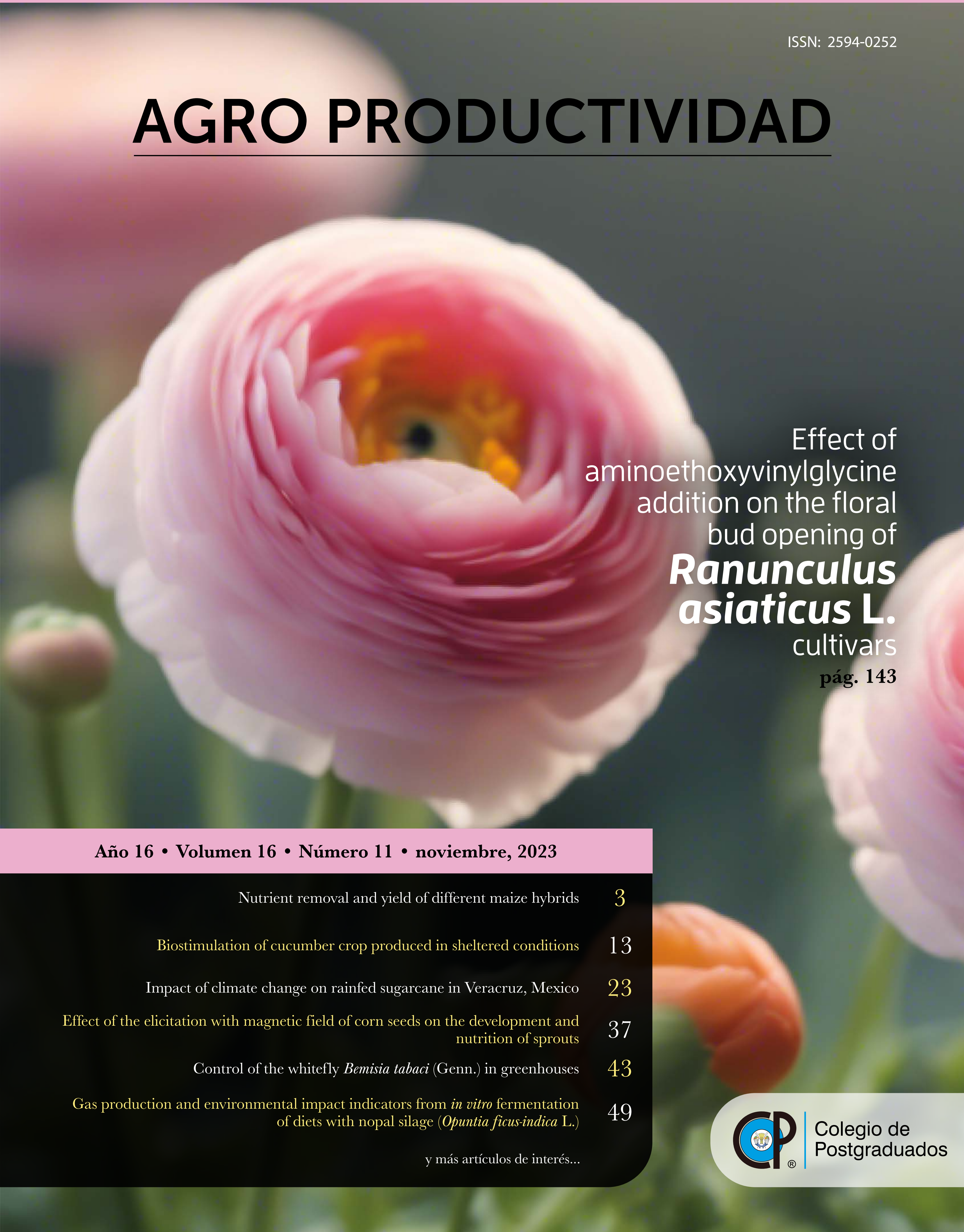

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Palabras clave y Keywords: Se deberá incluir una lista de 3 a 5 palabras clave en español e inglés que permitan identificar el ámbito temático que aborda el artículo.
Introducción: Se asentará con claridad el estado actual del conocimiento sobre el tema investigado, su justificación e importancia, así como los objetivos del trabajo. No deberá ser mayor a dos cuartillas.
Materiales y Métodos: Se especificará cómo se llevó a cabo la investigación, incluyendo el tipo de investigación, diseño experimental (cuando se traten de investigaciones experimentales), equipos, substancias y materiales empleados, métodos, técnicas, procedimientos, así como el análisis estadístico de los datos obtenidos.
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Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.
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Nutrient removal and yield of different maize hybrids

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ABSTRACT

Objective: to determine the macro and micronutrient removal values and yield of different hybrids, and, also to determine the relationship between grain nutrient removal and grain yield.

Design/methodology/approach: to assess correlations and determine the association degree between the nutrient removal values and grain yield.

Results: total nutrient removal values were N>K>Ca>Mg>P, and Mn>Fe>Zn>B>Cu, which were also higher when compared to other findings. Also, these values provide the mineral content in grains, which are nutritional quality parameters.

Limitations on study/implications: more hybrids, different fertilization rates, different soil conditions and crop management practices should be evaluated to assess whether or not they influence/inhibit grain nutrient concentration and total removal.

Findings/conclusions: The total grain nutrient removal values varied as a function of hybrids, yield goal, and nutrient concentration in tissues.

These values allow the adjustment of current fertilization rates. The same hybrids under different management practices (fertilization rate), or soil types, substantially influence the grain nutrient concentration and therefore total nutrient removal.

Keywords: nutritional extraction, fertilization, yield.

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INTRODUCTION

Agriculture is associated with environmental variables such as temperature, precipitation, and solar radiation. These variables, especially temperature increase affect crop development, water, and nutrient uptake rate, yield, and grain nutrient concentration. Maize (*Zea mays* L.) crop in the state of Sinaloa, Mexico, is one of the most important, not only for its planted area in each growing season but also for its nutritional value (SIAP 2023). According to several reports, maize's yield potential has been maximized due to (breeding) and the implementation of more efficient agronomic practices, but nutrient management is also essential. In that sense Ma *et al.* (2006), Ciampitti and Vyn (2013) Caires and Milla

(2016) showed that climatic variables and agronomic management directly impact grain nutrient removal; while the uptake and extraction processes associated with optimal yield improve fertilization management considering climate variability (Ciampitti and Vyn, 2011; Hill and Clérici, 2013). Currently, there is limited information on grain nutrient removal in maize seeds grown in semi-arid climate conditions of northern Sinaloa. Much of the existing information is outdated, from hybrids grown in other regions, and not useful for current hybrids, agronomic management practices, and climatic or edaphic conditions. Therefore, the objective of this research was to determine removal values of macro and micronutrients, yield potential of different maize hybrids, and the relationship between nutrient removal and grain yield.

MATERIALS AND METHODS

Description of the study area

The research was carried out during the autumn-winter growing season 2020-2021 in three different plots. The soils in the region are classified as clay loam (50% clay, 30% silt, and 20% sand), low organic matter content (<1%), bulk density of 1.15 g cm^{-3}, 48% field capacity, and 33% permanent wilting point.

Crop establishment

Planting was realized on moistened soil with a density of 90,000 plants. The hybrids, Dekalb 4055®, P3230W®, Asgrow Hipopótamo®, and P3274W® were planted from November 10 to 30, 2021, corresponding to the optimal planting window (INIFAP-CEVAF).

Crop fertilization

Fertilization was applied in the following stages: 400 kg ha^{-1} of bulk blend (30-10-12) pre-plant applied. The second fertilization event was done at V6 growth stage with 300 kg ha^{-1} of urea or anhydrous ammonia (NH_3), and a last application was done during the flowering stage (R1) with 100 kg ha^{-1} of NH_3.

Seed sampling nutrient

Seed samples were collected from each hybrid (100 g of seeds) at physiological maturity to determine nutrient concentration.

Grain drying

The 100 g of grain were dried in a forced air oven at 70 °C temperature for 24 hours, once dried, 20 g were taken for nutrient concentration.

Nutrient concentration in grain

Nutrient levels were estimated following the methodology described in the Official Mexican Standard (NOM-021-RECNAT-2000).

Total grain nutrient removal was estimated with Equation (1).

$$\text{Nutrients in grain}\left(\text{kg ha}^{-1}\right)=\left[\text{ yield}\left(\text{kg ha}^{-1}\right)*\%\text{ nutrient}/100\right] \quad (1)$$

Yield evaluation

For yield evaluation, sampling of each hybrid was assessed in a 3 m^2 area, considering the two central rows where cobs were collected. Subsequently, the moisture content in grains was measured with a humidity tester (Agratronix Mt-pro). The humidity adjustment to 14% of the grain was carried out with Equation 2:

$$HG(14\%)=WG\text{ kg}*(100-\%HG)/86 \quad (2)$$

Where: *HG* is the humidity of grain adjusted to 14%; *WG* is the weight of the grain (kg); %*HG* is the grain moisture percentage; 86 is the factor to standardize the yield at 14% humidity.

To estimate the yield components, cob length, number of rows, number of grains per row, cob weight (including its core), grain weight per cob, and weight of 100 grains were quantified. Finally, grain yield was calculated with Equation 3.

$$Y\left(\text{t ha}^{-1}\right)=(WG\text{ 14\% kg})*\left(HA\text{ m}^2/SHA\text{ m}^2\right)/1000 \quad (3)$$

Where: *Y* is the grain yield (t ha^{-1}) *WG* 14%: grain weight *SH* is the area of 1 hectare (m^2); SHA is the area of sampling (m^2).

Statistical analysis

Data of yield and its components were analyzed with one way anova. Regression models were fitted and tested on significance levels (α=0.05) and R^2 values using the data of nutrient concentration (Minitab, 2017).

RESULTS AND DISCUSSION

Yield and its components

Table 1 shows the values of yield and total yield components for each of the evaluated hybrids. No statistical differences were found between cob length and the number of rows among the hybrids. However, numerically the P3230W® and P3274W® hybrids reported higher cob weight and number of grains per cob.

Statistical differences were found regarding the weight of 100 grains, since the highest weight was obtained from the hybrid P3274W® and the lowest from Asgrow Hipopótamo®, with an average of 55 g.

Duarte *et al.* (2019) reported average values of 33.2 and 34.6 g in different hybrids. It is worth mentioning that all the yield component values represent the average of the hybrids grown in the same area and the same growing season.

The highest yield was found on P3274W® while the lowest yield on Dekalb 4055® (Figure 1). In this sense, Machado-Silva *et al.* (2018) reported similar yield values on hybrids DKB

Table 1. Yield components in maize varieties.

Hybrid	EL	RN	G/R	EW (kg)	GW/E (kg)	Weight of 100 grains (gr)
DK 4055	16.81 (±1.78) a	16.19 (±0.95) a	32 (±2.44) b	0.197 (±0.004) b	0.182 (±0.017) b	51 (±0.003) c
P3230W	16.73 (±2.08) a	16.02 (±0.24) a	33 (±2.22) ab	0.211 (±0.008) a	0.183 (±0.033) a	60 (±0.005) b
Hipopótamo	16.50 (±1.90) a	15.59 (±1.71) a	35 (±5.34) a	0.198 (±0.016) b	0.195 (±0.019) b	38 (±0.003) d
P3274W	16.43 (±1.75) a	14.90 (±0.82) a	34 (±2.46) a	0.215 (±0.004) a	0.200 (±0.014) a	72 (±0.003) a
Mean	16.6	15.6	33.5	0.205	0.19	55
(P≤0.05)	0.554	<0.0001	<0.0001	0.016	0.021	<0.0001

EL: ear length, RN: row number, G/R: grains per row, EW: ear weight, GW/E: grain weight per ear. Means with distinct letters within a column are statistically different (Fisher LSD P≤0.05), ±standard deviation.

310PRO2 (13.219 kg ha^{-1}), DKB 390PRO (10.793 kg ha^{-1}), AG 8088PROX (10,784 kg ha^{-1}) and P30F53 YH (9.299 kg ha^{-1}) grown in contrasting environments of Brazil.

Nutrient concentration in grain

The nutrient concentration present in grain was in the following order: N>K>Ca>Mg>P with average values of 11.5, 10, 6, 5, and 3.4 g kg^{-1} of seed. The micronutrients concentration was Mn>Fe>Zn>B>Cu with mean values of 415, 436, 172, 104, and 33 mg kg^{-1} (Table 2). Duarte (2003) reported values of 13.7, 3.6, 4.7, 0.1 1.3, 1.0 g kg^{-1} of N, P, K, Ca, Mg, S, and 32.3, 8.1, 4.0, 30.1, 6.0 mg kg^{-1} of Fe, Mn, Cu, Zn, and B respectively. The micronutrient values are lower than those found in this research. However, P, K, and Mg concentrations are higher than those reported by Duarte *et al.* (2019) who found nutrient concentration of N>K>P>Mg>S>Ca>Zn>Fe> Mn>B>Cu with mean values of 13.1, 3.1, 2.1, 1.1, 0.9, 52.5 g kg^{-1} and 17.5, 13.1, 4.7, 3.7, 1.8 mg kg^{-1} in different hybrids and locations. They also coincide with findings reported by Resende *et al.* (2012) who showed an approximate range values of N (15.7 g kg^{-1}), P (3.1 g kg^{-1}), K (3.7 g kg^{-1}), and Oliveira Junior *et al.* (2010) who reported a slight decrease in P concentration (2.4 g kg^{-1}).

Research by Heckman *et al.* (2003) found that maize hybrids evaluated over 23 years and different sites, exhibited a wide range of macronutrient concentrations: N (10.2-15.0 g kg^{-1}), P (2.2-5.4 g kg^{-1}), K (3.1-6.2 g kg^{-1}), S (0.9-1.4 g kg^{-1}), Mg (0.88-2.18 g

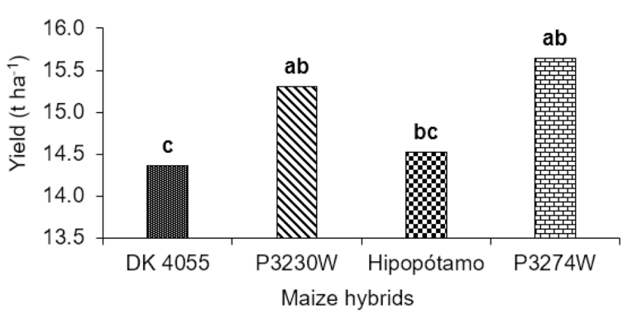

Figure 1. Yield of evaluated maize hybrids.

kg^{-1}), and Ca (0.13-0.45 g kg^{-1}) and that micronutrients had a greater variation in these concentrations, which affected the total nutrient removal. On the other hand, Bender *et al.* (2013) reported concentrations of N (13.8 g kg^{-1}), P (3.3 g kg^{-1}), K (4.4 g kg^{-1}), Mg (1.4 g kg^{-1}), Fe (21 mg kg^{-1}), Mn (6.0 mg kg^{-1}), Cu (3.4 mg kg^{-1}), Zn (26 mg kg^{-1}) and B (1.6 mg kg^{-1}) in six maize hybrids. Finally, Binford (2010) mentions that average N concentration ranges between 12 and 15 g kg^{-1}, while P and K range between 3.0 and 3.6 g kg^{-1}.

The existing variability values found in this study as compared to other research can be mainly attributed to climatic and edaphic conditions, length of growing season, and agronomic management.

In this sense, various authors such as Ciampitti and Vyn (2013) and Caires and Milla (2016) argue that nutrient content in grains is strongly influenced by the diversity of materials and agronomic management.

Total nutrient removal

According to Table 2, P3274W® hybrid reported the highest total nitrogen removal (219 kg ha^{-1}) compared to DK4055®, which had the lowest removal (130 kg ha^{-1}). P3230W® and Asgrow Hipopótamo® showed similar removal values of 179 and 161 kg ha^{-1}. The average N removal among the evaluated hybrids was 172 kg ha^{-1}. Phosphates $\left(PO_4^-\right)$ removal showed a similar trend to that of nitrogen, where the highest removal occurred in P3274W® and the lowest in DK4055® and Asgrow Hipopótamo® with 36 and 34 kg ha^{-1}.

In the same manner, P3274W® and P3230W® showed the highest removal as compared to DK4055® and Asgrow Hipopótamo® with 120 and 124 kg ha^{-1}. The average PO_4^- removal was 52 kg ha^{-1}. K^+ and Mg removal had a very similar trend in the evaluated hybrids. P3274W® and P3230W® showed the highest removal with 184-172 kg ha^{-1} of K and 109-194 kg ha^{-1} of Mg. While DK4055® and Asgrow Hipopótamo® extracted approximately 124-120 kg ha^{-1} of K and 47-54 kg ha^{-1} of Mg. The average K removal was 150 kg ha^{-1} and 76 kg ha^{-1} for Mg.

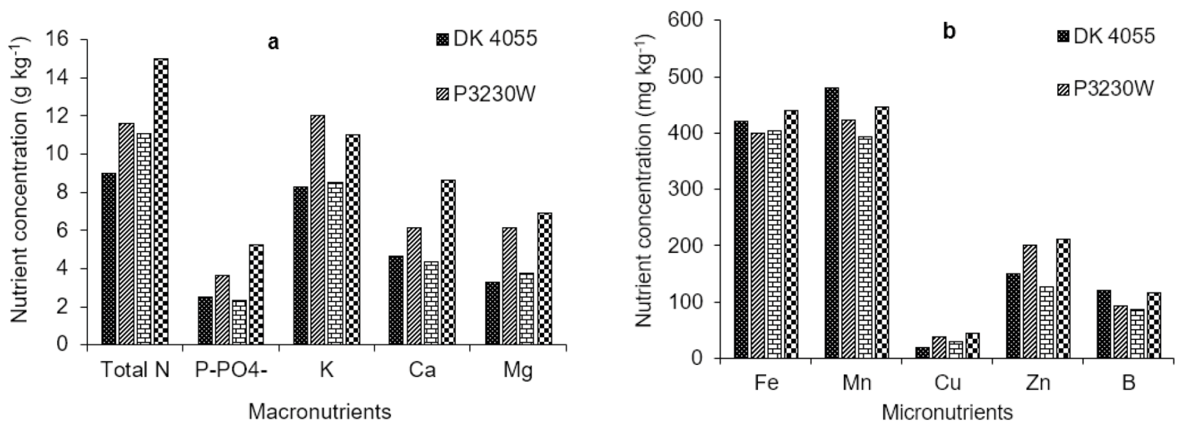

Figure 2. Grain nutrient concentration of different maize hybrids, Macronutrients (a), Micronutrients (b).

Calcium removal was greater in P3274W® with 135 kg ha^{-1} but numerically different than DK4055® and Asgrow Hipopótamo® with 67 and 63 kg ha^{-1} respectively. The average removal was 89.5 kg ha^{-1}. Iron and manganese removal in the evaluated hybrids was similar. However, P3274W® and P3230W® had numerically higher removal values compared to the other hybrids. The highest copper removal was observed in P3274W® with 69.4 g ha^{-1}, followed by P3230W® with 59 g ha^{-1}, Asgrow Hipopótamo® with 43.5 g ha^{-1} and finally DK4055® with 29 g ha^{-1}.

Higher zinc removal, (331 g ha^{-1}) occurred in P3274W®; while Asgrow Hipopótamo® extracted the least amount (182 g ha^{-1}). Finally, no variability was observed on boron removal values for P3274W® and DK4055® with 183 and 172 g ha^{-1} compared to Asgrow Hipopótamo® with 126 g ha^{-1}.

Overall, the average macronutrient removal among the evaluated hybrids in this research was comparable to those found in other studies, except for some that exceeded the accumulation range, such as K removal with 150 kg ha^{-1}, Mg with 76 kg ha^{-1} and Ca with 89.5 kg ha^{-1}.

According to Bender *et al.* (2012), the average nutrient removal values found in six transgenic hybrids grown in two locations were 166, 90, 66, and 17 kg ha^{-1} of N, P_2O_5, K_2O, Mg and 248, 72, 41, 166 and 19 g ha^{-1} of Fe, Mn, Cu, Zn, and B respectively. However, the same authors emphasize that environmental conditions of the sites where crops are grown strongly influence the total nutrient removal.

In other research, Heckman *et al.* (2003) found macronutrient removal range of 145-188, 73-108, and 57-78 kg ha^{-1} of N-P-K respectively. While, Duarte *et al.* (2019) reported removal values of 157-232, 50-80, 40-55, 12-16, 0.3, and 0.4 kg ha^{-1} for N-P-K-Mg-Ca for different hybrids. Finally, Sifuentes *et al.* (2015) evaluated Pioneer P3245W® under two low-pressure irrigation systems (PVC pipe - lay flat hose) and surface irrigation (furrows) at Valle del Fuerte reporting the following nutrient removal values: N (165, 173, and 138 kg ha^{-1}), P (4.4 and 6 kg ha^{-1}), K (11, 10 and 9 kg ha^{-1}), Ca (9, 11 and 7 kg ha^{-1}), Mg (19, 13 and 7 kg ha^{-1}) for each of the evaluated systems.

Nutrient removal as a function of yield

The analyzed nutrients were correlated with yield, finding significant differences. In all cases, the correlation coefficients were in ranges above R^2=0.80, except for Ca removal (R^2=0.45). Hence, nutrient removal strongly varies with respect to yield, even when the coefficient of determination (R^2) is low.

Nitrogen removal values were in the range of 126 to 240 kg ha^{-1} for yields of 14 and 16 t ha^{-1} (Figure 3a). These concentrations significantly impact the protein content in grains, which represents a good quality parameter. PO_4^- removal was in the range of 30 to 50 kg ha^{-1} with yields between 14 and 15 t ha^{-1} (Figure 3b). The same trend was observed in K removal (133 and 180 kg ha^{-1}) with an average yield of 14.5 t ha^{-1}, up to 190 kg ha^{-1} when the yield approached to 16 t ha^{-1} (Figure 3c).

Calcium extraction was in the range of 78 to 119 kg ha^{-1} (Figure 3d); while Mg ranged between 50 and 118 kg ha^{-1} for the same yields (Figure 3e). Different studies mention that

Table 2. Total nutrient removal of different maize hybrids.

Hybrid	Macronutrients						Micronutrients			
	N	P - PO_4^-	K^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	Mn^{2+}	Cu^{2+}	Zn^{2+}	B
	kg ha^{-1}						g ha^{-1}			
DK 4055	130	36.0	120	67	47	602	689	29	216	173
P3230W	179	55.5	184	93	94	616	650	59	309	143
Hipopótamo	161	34.0	124	63 b	54	586	570	43.5	182	126
P3274W	219	82.5	172	135	109	691	699	69.4	331	183
Mean	172	52.0	150	89.5	76	623	652	50	259.5	156

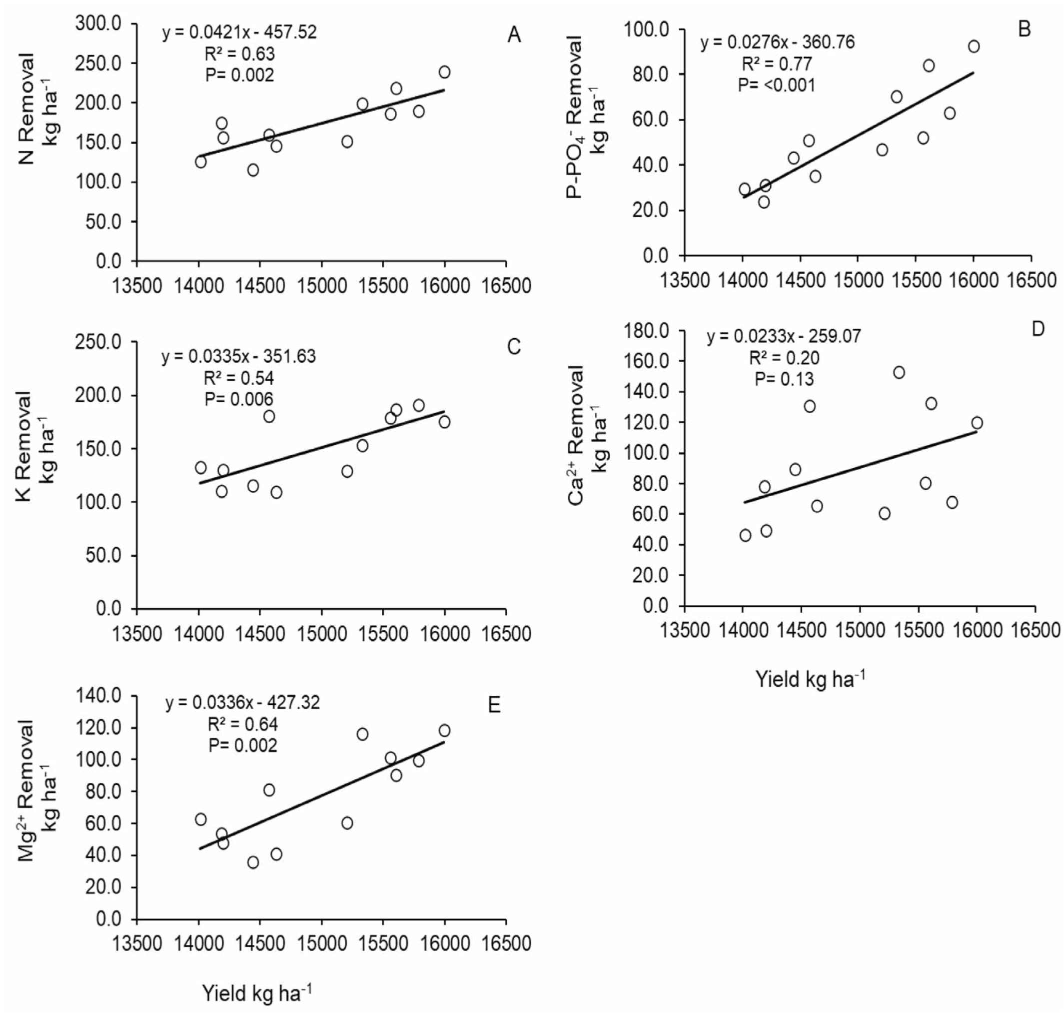

Figure 3. Total nutrient removal of N (a), P (b), K (c), Ca (d), and Mg (e) as a function of yield.

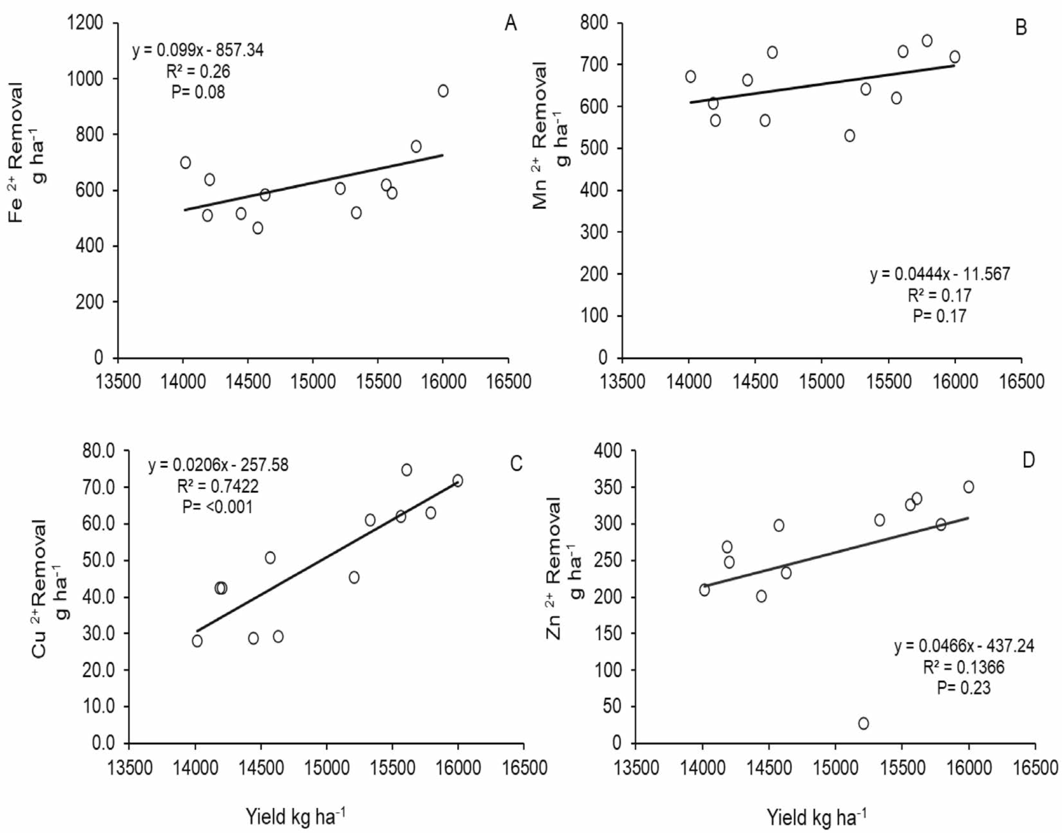

Figure 4. Total nutrient removal of micronutrients Fe (a), Mn (b), Cu (c), and Zn (d) as a function of yield.

nutrient removal in grain is the product of their concentration in that organ, as well as the yield, and agronomic practices (plant density, hybrid).

In that sense, findings reported by Below *et al.* (2010) mention that transgenic hybrids (with insect protection against cutworms and corn borers) increased the nutrient removal of N, P, K, S, and Zn, arguing that a greater influence was observed on the immobile nutrient uptake than on mobile ones. Similarly, Bender *et al.* (2012) found a removal range of N (145-188 kg ha^{-1}), P (73-108 kg ha^{-1}), K (57-78 kg ha^{-1}), Mg (15-20 kg ha^{-1}), Fe (218-285 g ha^{-1}), Mn (62-87 g ha^{-1}), Cu (30-49 g ha^{-1}), Zn (269-353 g ha^{-1}) and B (13 -32 g ha^{-1}) for an average yield of 12 t ha^{-1} in transgenic hybrids.

Finally, Raymond *et al.* (2009) mention that nutrient uptake and grain concentration decrease by increasing plant density, despite yield increase.

CONCLUSIONS

This research found that nutrient removal in grain varies as a function of hybrids, yield goal, and nutrient concentration in tissues. The removal values are higher as compared to other studies. Therefore, they are useful for making adjustments in fertilizer rates and application times. This research demonstrates that planting the same hybrids under different agronomic management conditions (fertilization rates) or soil types can substantially influence the nutrient concentration in grain and, consequently the total nutrient removal values.

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Colegio de Postgraduados

Biostimulation of cucumber crop produced in sheltered conditions

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ABSTRACT
Objective: To evaluate the physiological and productive response of cucumber crop to the induction of three commercial phytohormones (Cito Xplosión, Súper Hormonal, Vitaminum Forte) grown under protected conditions.
Design/methodology/approach: The experiment consisted of three treatments, T1 (Cito Xplosión), T2 (Súper Hormonal), and T3 (Vitaminum Forte). The evaluated variables were plant height, number of leaves, fresh biomass, fruit diameter and length, number and weight, total soluble solids, pH, and electrical conductivity.
Results: In the physiological variables, values of plant heights were 225, 228, 220, and 238 cm for T1, T2, T3, and the control. 33 leaves for all treatments and 31 for the control, T1 and T2 produced higher fresh biomass in leaves, stems, roots, and flowers, while T3 produced higher biomass in fruits. For the fruit length, the values were 16.9, 17.6, 17.6, and 18.4 cm, and diameters of 5.2, 5.2, 5.1, and 5.1 cm for T1, T2, T3, and the control. The weight of fruits was 293.2, 297.3, 283.9, and 281.7 g with yields of 10.1, 10.9, 10.0, and 9.6 kg m^{-2} for T1, T2, T3, and the control.
Limitations on study/implications: More varieties should be evaluated using different nutrient solution concentrations and management practices as a function of the number of stems to assess whether phytohormones influence physiological or productive variables.
Findings/conclusions: From an economic point of view, T2 was the best treatment, achieving a higher yield and fruit quality.

Keywords: phytohormones, growth and development, quality and yield of fruits.

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the best-known cucurbit vegetables. It is almost grown worldwide, mainly for fresh consumption in an immature state (Barraza-Álvarez, 2015). Cucumber consumption is ranked as the fourth most important vegetable in the world, after tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L. var. *capitata*), and onion (*Allium cepa* L.) (Barraza-Álvarez, 2015).

Actually, there are a series of inputs that improve the growth and development of crops, among them are phytohormones, which are natural or synthetic compounds that affect metabolic processes and may improve the productivity and quality of fruits (Gollagi *et al.*, 2019). These compounds are molecules synthesized by the plant that control the vast majority of physiological and biochemical processes such as cell division, growth, and differentiation of aboveground organs and roots (Porta and Jiménez-Nopala, 2019).

The use of phytohormones has made it possible to specifically control processes such as production of secondary metabolites, growth time, reduction of pathogenic agents, fruit ripening induction, and breeding of plant species to improve industrialized products, which are difficult to regulate in a conventional production system (Vega-Celedón *et al.*, 2016).

One of the currently applied tools in agriculture is using phytohormones or plant growth regulators. The application of these compounds modifies plant development to induce thinning, flowering, fruit set, size, and uniform ripening of fruits. The objective of this research was to evaluate the physiological response, production, and biochemical components of cucumber crops with the induction of three commercial plant phytohormones in protected conditions.

MATERIALS AND METHODS

Experiment description

The experiment took place at Colegio de Postgraduados, Montecillo Campus, Estado de Mexico (19° 27' 58" North latitude and 98° 54' 58" West longitude, and 2431 m altitude). Seeds of Poinsett 76 variety (indeterminate growth habit) were germinated in expanded polyethylene trays with 200 cavities. They were sown on July 23, transplanted on August 26 until November 25, 2021. The material was grown in a polycarbonate greenhouse under hydroponic system, in black polyethylene bags (12 L) with red tezontle as substrate.

The planting method was the triangular system "tresbolillo" with 40 cm apart from each plant and between lines. They were planted in twin lines (20 m long) with a density of three plants per m^2. Every eight days, lateral shoots were pruned with T-67 pruning shears to keep one-stem plants. The drip irrigation system consisted of a watering line (16 mm diameter) with self-compensated drippers (0.4 m apart), a flow rate of 8 L h^{-1}, and an operating pressure of 68.64 kPa.

Irrigation

Irrigation was applied with a Steiner (1984) nutrient solution of −0.087 MPa osmotic potential and pH 6.5 throughout the growing season. A flow rate of 0.18 L $plant^{-1}$ day^{-1} was applied during the first 30 days after transplant, which corresponded to the initial stage, 0.380 L during vegetative stage, 0.50 L $plant^{-1}$ in development stage, 0.60 L in production stage (peak demand) and 0.52 L at the end of the season.

Treatment description

Three treatments (T) and a control (CON) were established, which consisted of the application of three commercial phytohormones: T1 (Cito Xplosión®), T2 (Super

Hormonal®), and T3 (Vitaminum Forte®) (AGRONORTECH company) and control without application. The rate applied was 3 mL L^{-1} during the flowering stage, and fruit set; and 5 mL L^{-1} during fruit formation, fruit filling, and beginning of harvest, no application was supplied to the control.

Experimental Design

Each experimental unit was 20 m^2 with 15 plants and 4 replicates and a total area of 80 m^2 per treatment on a randomized complete block design. Treatment mean differences were separated using Tukey least significant difference (LSD) test at $p \leq 0.05$ using MINITAB® release 16 Statistical software.

Response variables

Plant height PH (cm): measured from the base to the apex. Stem diameter SD (cm): measured with a vernier caliper, 1 cm from the base of the plant. Number of leaves per plant. Number of fruits per plant (NFP) during production, number of fruits per plant at physiological maturity. Fresh matter: destructive sampling of plants was carried out. This consisted of extracting the plant from the pot and separating organs (leaves, stems, fruits, and roots). Subsequently, these were weighed fresh and placed in a drying oven (70 °C) for 72 h until constant weight.

Fruit size classification

Four categories were used according to Mexican standard (NMX-FF-023-1982) (Table 1). Fruit length was obtained with a measuring tape (model 32G-8025). The diameter was determined with a vernier (Truper CALDI-6MP).

Biochemical components evaluation

Total soluble solids (TSS): determined from the fruit juice with a Hanna model HI96801 refractometer and expressed in °Brix. Fruit firmness was measured with a FDV30 texturometer (Greenwich, CT 06836, USA), recording the skin resistance to puncture and expressed in newtons (N). The pH and electrical conductivity (EC) were directly determined in the fruit juice (Hanna instruments-model HI98130), the EC values were expressed in dS m^{-1}.

RESULTS AND DISCUSSION

Evaluation of physiological variables

Plant height

Figure 1 shows that growth slowly begins until 30 days after transplant (DAT). Subsequently, at 37 and 51 DAT, development noticeably increased in all treatments until reaching a height of 116, 115, 112, and 98 cm for T1, T2, T3, and control (Figure 1). At 58 DAT, due to the effect of the phytohormones, it was observed that plants regulated their growth speed since a part of their energy was translocated to flowering, pollination, and fruit set. The opposite occurred in the control treatment, as the plant used its energy in growth, regardless of fruit production.

Table 1. Size classification of cucumber fruits according to Mexican standard.

Quality	Diameter (cm)	Length (cm)
Big	>6.5	>16.5
Medium	5.1-6.5	15.1- 16.5
Small	3.5- 5.0	14.0- 15.0
Lag	>3.4	>14.0

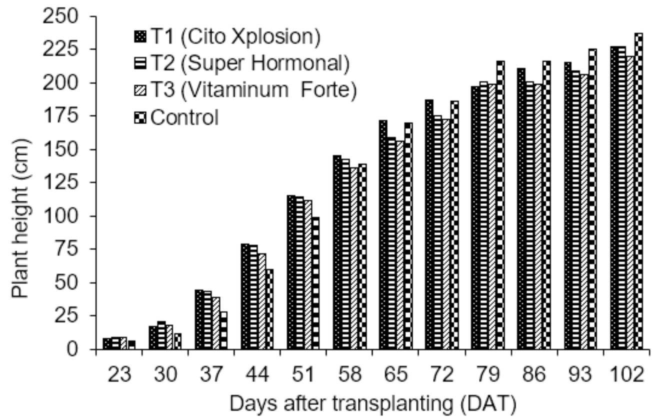

Figure 1. Plant height for evaluated treatments.

The maximum height was 227, 228, 220, and 238 cm for T1, T2, T3, and control (Figure 1). Furthermore, at 65 DAT, control treatments significantly began to accelerate its growth compared to the rest of the treatments. The values reported here are higher than those reported by Gabriel-Ortega *et al.* (2022) who found 161-80 cm height due to the application of different types of biostimulants in cucumber crops grown under greenhouse conditions.

The phytohormones applied in all treatments were statistically significant ($p \leq 0.05$) in the variables of fruit weight, yield, firmness, and fruit diameter. However, in plant height, no statistical difference was found between the treatments compared to the control (Figure 1).

Stem diameter and number of leaves

There were no significant differences between the treatments ($p \geq 0.05$) with respect to stem diameter. The results in this research were on average of 1.1 cm, which agree with those reported by Ayala-Tafoya *et al.* (2019) who found a stem diameter of 1.07 cm in cucumber plants "Alanis RZ F1" variety planted at a density of 1.68 plants m^2 and pruned to one stem per plant. Gabriel-Ortega *et al.* (2022) reported a 0.98 cm stem diameter in the variety "Intimator". This morphological trait has shown greater genetic propensity since Ortiz *et al.* (2009) reported differences in stem diameter (0.61 - 0.77 cm) between cucumber varieties.

33 leaves per plant were obtained in the three treatments and 31 leaves in the control, with no significant differences due to application of phytohormones. Ayala-Tafoya *et al*. (2019) reported 41 total leaves and 260 cm height in a cucumber crop grown in a greenhouse. The number of leaves is directly related to the leaf area. Therefore, they are important parameters in plant growth evaluation and their determination is essential for the correct interpretation of physiological processes in plants (Mendoza-Pérez *et al*., 2018b; Ayala-Tafoya *et al*., 2019).

Fresh biomass

The importance of evaluating fresh biomass in plants relies on the quantitative determination of water content (González *et al*., 2018). A similar accumulation was obtained in all treatments compared to the control during the initial stage. However, starting at 51 days after transplant (DAT), the fresh biomass accumulated in leaves, stems and roots started to increase. At 72 DAT, fruit production increased in all treatments along with the control, which corresponded to the stage that harvest began.

The performance of the three treatments was similar (Figure 2). Furthermore, it is important to know this variable because it can serve as a tool in irrigation and fertilization

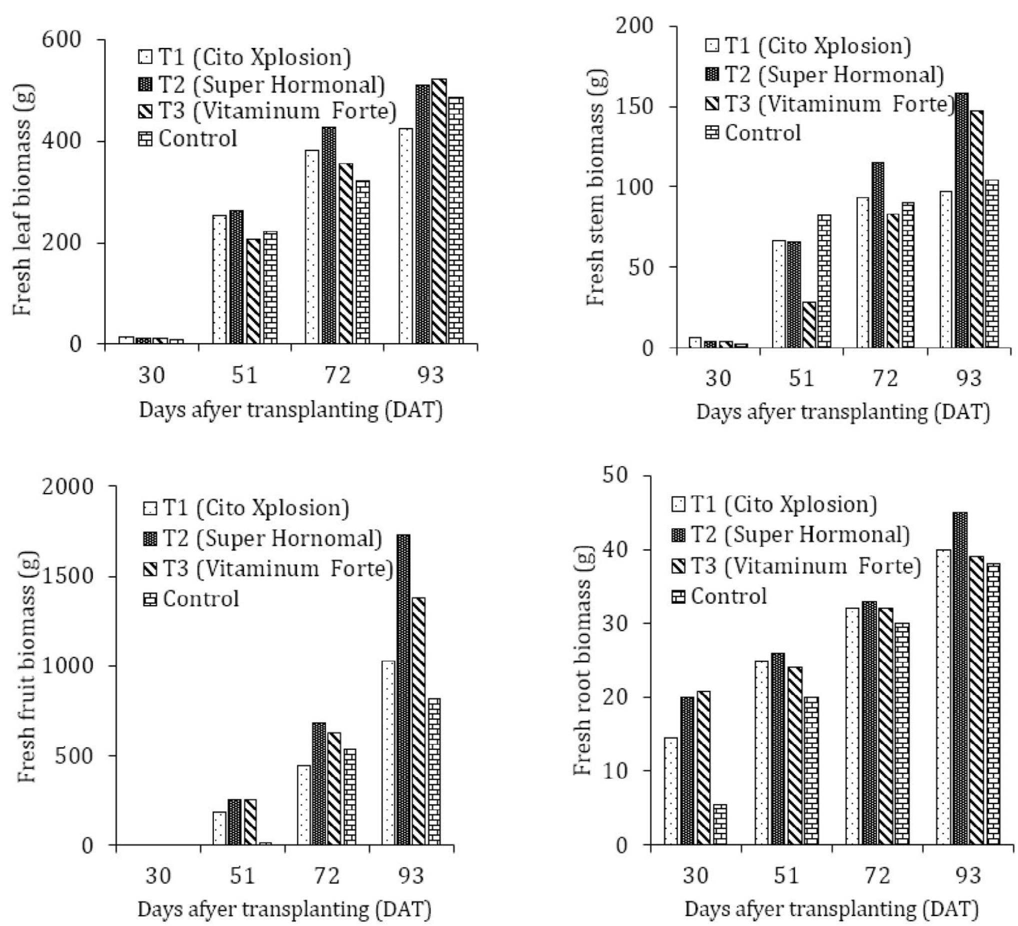

Figure 2. Fresh biomass accumulation in plant organs of all treatments.

scheduling on this crop. In the case of fruits (Figure 2), at 51 DAT the fresh biomass accumulation started to increase in all treatments including the control.

Physical characteristics and fruit yield

Fruit length

The length and diameter variables of fruits are components related to quality attributed to the size and fruit appearance. These are the most important parameters considered when classifying the size of the fruits for export. In this research, values of 16.9, 17.6, 17.6, and 18.4 cm in length were found for T1, T2, T3, and control respectively, with no significant differences due to the phytohormones. This response was also found in fruit diameter. Montaño *et al.* (2018) reported a value of 17.5 cm in cucumber fruit, cultivar Poisentt 76.

Fruit diameter

In this research, fruit diameters of 5.2, 5.2, 5.1, and 5.1 cm were found for T1, T2, T3, and control respectively. Montaño *et al.* (2018) reported a fruit width of 5.0 and 6.0 cm in Poisentt 76 cultivar, while López-Elías *et al.* (2011b) reported 5.0 cm diameter. Therefore, the values reported here are within the established range of 5.0 cm for American-type cucumber. A positive effect was found in this variable, given that treatments with applied phytohormones increased fruit diameter (1 cm) compared to the control treatment. According to various researchers, fruit diameter ranges between 4.3 and 5.3 cm for large cucumbers. Westwood cited by Montaño and Méndez (2009) pointed out that fruit width depended on other parameters such as the cortical area, pulp, and central cavity.

Average fruit weight

The average weight of cucumber fruits is presented in Table 2. T2 (Super hormonal®) presented the highest numerical value (297.3 g) compared to the rest of the treatments. Montaño (2018) obtained a similar results (293 and 317 g) average weight of cucumber fruits in Poinsett 76 cultivar. Chacón *et al.* (2017) reported weights of 224-239 g for three cucumber genotypes grown in greenhouse conditions. Chacón-Padilla and Monge-Pérez (2020) reported a fruit weight of 278 g, while Sánchez del Castillo *et al.* (2014) reported 270 g in Alcázar cultivar grown in hydroponic system.

Table 2. Stem diameter, number of leaves, length, and fruit diameter.

Treatments	NF m^2	FD (cm)	FL (cm)	MWF (g)	FY ($kg\ m^{-2}$)
T1 (Cito Xplosión)	36	5.2 a	16.9 a	293.2 b	10.1 b
T2 (Súper Hormonal)	36	5.2 a	17.6 a	297.3 a	10.9 a
T3 (Vitaminum Forte)	36	5.1 a	17.6 a	283.9 c	10.0 b
Control	33	5.1 a	18.4 a	281.7 d	9.6 c

Columns with distinct letters are statistically different. Fisher/Tukey Mean Separation Test ($P<0.05$). NF: number of fruits, FD; fruit diameter, FL; fruit length, MWF; medium weight of fruits, FY; Fruit yield.

Number of fruits

No statistical difference was found in the number of fruits per plant due to application of phytohormones in all treatments. Sánchez del Castillo *et al.* (2014) reported 38.5 fruits m^2 in Alcázar cultivar with a density of 6 plants m^2 grown in a greenhouse in different hydroponic systems.

Golabadi *et al.* (2013) reported that the number of cucumber fruits per plant projects the greatest positive effect on the total production, indicating that this parameter is one of the most reliable components for selecting genotypes with high fruit yield. Chacón-Padilla and Monge-Pérez (2020) reported 18.83 fruits per plant and mentioned that large sized fruits reached a greater length and fruit weight, taking approximately 15 days to develop each fruit. Therefore, larger size reduced the plants' ability to produce a greater number of fruits per plant.

Fruit yield

Values of 10.1, 10.9, 10.0, and 9.6 kg m^{-2} for fruit yield were obtained for T1, T2, T3, and control respectively. These results demonstrate that the correct use and application of plant phytohormones in peak demand stages of cucumber cultivar can be a viable alternative to improve fruit quality and yield. Chacón-Padilla and Monge-Pérez (2020) reported similar yield of 8.7 kg per plant. According to the results obtained in this research, a balanced concentration of the nutrient solution and the application of biostimulants in stages of maximum water and nutrient demand can be the key to improving the growth processes, development, and formation of organs, quality, and fruit yield.

Fruit size classification

In the fruit size by length classification, the control treatment showed higher values (84, 2, 11, and 2% large fruit, medium, small, and lagging fruit), while T2 (Super hormonal®) had fruits of 82, 6, 12 and 0% large, medium, small and lag respectively.

Regarding its diameter, T1 (Cito Xplosión®) had the highest value of 7, 52, 41, and 0% large, medium, small, and lag fruits. The effect of applying phytohormones in the treatments increased the harvested fruit diameter compared to the control. The results found in this research coincide with those reported by Reyes-Pérez *et al.* (2019) who found significant differences in polar and equatorial diameter in Hybrid SARIG 454 with the application of chitosan biostimulant (200 mg ha^{-1}).

Biochemical components

The firmness variable was significantly different in all treatments. T3 (Vitaminum Forte®) had the highest value of 6.2 N. This treatment accumulated less fresh matter which was mainly attributed to the lower biostimulant concentration. In the case of the soluble solids, no significant differences were found between the treatments. However, numerically, T3 (Vitaminum Forte®) was the highest with 6.2 °Brix. Cucumbers are non-climacteric fruits characterized by low total soluble solids, so the sugar accumulation during the growth and maturity stage does not experience significant changes. Moreno-Velázquez *et al.* (2013)

reported values of 3.75 °Brix for Zapata cultivar, 3.47 for Lider cultivar and 2.95 °Brix for Constable cultivars.

The pH values in fruit juice were 4.9, 4.2, 5.3, and 5.2 for T1, T2, T3, and control respectively (Table 3). Moreno-Velázquez *et al.* (2013) reported higher pH values (5.64) for Zapata cultivar, 5.94 for Lider and 5.6 for Constable cultivar). The pH is a measure of H+ ions concentration in any aqueous solution. Therefore, low pH values indicate a higher concentration of H+ ions and vice versa. Regarding the EC variable, T1 presented the highest value (3.0 dS m^{-1}).

Table 3. Biochemical components in cucumber fruit juice.

Treatments	Firmness (N)	°Brix	pH	Electrical conductivity (dS m^{-1})
T1 (Cito Xplosión)	4.8 b	5.8 a	4.9 b	3.0 a
T2 (Súper Hormonal)	4.4 c	6.0 a	4.2 b	2.9 b
T3 (Vitaminum Forte)	6.2 a	6.2 a	5.3 a	2.4 c
Control	4.1 c	6.1 a	5.2 a	2.9 b

Columns with distinct letters are statistically different. Fisher/Tukey Mean Separation Test ($P<0.05$).

CONCLUSIONS

The exogenous application of biostimulants had a positive effect on the vegetative development of plants and promoted the increase in morphological characteristics of the fruits, such as diameter, firmness, and weight. In biochemical components, biostimulants favored the total soluble solids accumulation. It is important to note that biostimulant induction in crops during key phenological stages substantially increases the plants' capacity to carry out photosynthesis, water, and nutrient uptake. With this technique, the yield and quality of crop fruits increase, contributing to the growers' economy and the population's nutrition.

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Impact of climate change on rainfed sugarcane in Veracruz, Mexico

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ABSTRACT

Objective: To estimate the expected quantitative changes of the rainfed sugarcane yield in four sugar mills in the state of Veracruz using climate change scenarios for the end of the 21st century and considering that the same climate change could also affect soil fertility.

Design/methodology/approach: The data on the cultivated area with the rainfed sugarcane such as topography, principal properties of soil fertility, crop yields for the beginning of the 21st century, current climatic data from the meteorological stations and future ones based on existing climate change scenarios were analyzed. Then, by means of a physiological model of this crop based on biological, climatic and soil characteristics, proposed by IIASA/FAO, the current and future agricultural productivity of sugarcane was calculated. The actual productivity calculated with this model was compared with the observed data. Then, the productivity of this crop for the end of the 21st century was calculated. The comparative impact on the productivity of the expected changes in some climatic components and corresponding expected modification in soil fertility was assessed.

Results: The results of calculations indicate that if the CO_2 concentration in the atmosphere increases by 2 or 2.7 times at the end of the 21st century and the current varieties of sugarcane and their crop management will conserve, the yield of sugarcane will decrease up to 20% depending on the climate change scenario and location of the plot. The main climatic factor influencing the decrease in sugarcane productivity is the expected decrease in precipitation.

Limitations on study/implications: Monthly average climatic variables are used for both current and future productivity calculations since there are no estimates of daily data. There are also no predictions on the development of crop management technology as well as on the expected change in pests and diseases for the end of the 21st century.

Findings/conclusions: The IISA/FAO physiological model of sugarcane growth based on agroecological principles, considering even limited number of climatic variables, is useful for calculating of sugarcane productivity with correlations greater than 90% for calculated and observed data. This allowed us to estimate the expected impact of climate change in the productivity of rainfed sugarcane in Veracruz State of Mexico at the end of 21st century.

Keywords: calculation of agricultural crop yield, radiative index of dryness, integral soil fertility index, scenarios of the climate change.

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INTRODUCTION

Sugarcane (*Saccharum officinarum*) is an important crop for Mexico. SIAP (2022) reported that the country ranked eighth worldwide in annual production of 55 million tons of sugarcane and that the state of Veracruz ranked first in the country with a production of 21 million tons. About 50% of this crop in the state of Veracruz is produced under rainfed conditions.

Despite the importance of this crop and the existence of programs to support producers to increase the sugarcane production, there are few studies on the impact of the global climate change on this crop. Studies carried out in several countries around the world indicate that climate change can significantly affect the productivity of rainfed sugarcane (Srivastava and Mahendra, 2012; Marin *et al.*, 2013; Guerra and Hernández, 2014; Zhao and Li, 2015; Linnenluecke *et al.*, 2018). These studies used different approaches such as: analysis of observed statistical data on historical crop yields depending on climatic conditions, econometric regression models, simple models of crop development, etc. However, there are no studies based on physiological modeling of the sugarcane growth. In addition, the alteration of soil fertility depending on the climate change and its indirect impact on crop productivity have not been considered. Meanwhile this indirect impact can cause change up to 30% in the productivity of some agricultural crops (Castillo *et al.*, 2007).

Therefore, the purpose of this paper is to estimate the impact of the global climate change on the productivity of rainfed sugarcane in the state of Veracruz by the end of the 21st century using a physiological model of sugarcane growth (IIASA/FAO, 2012). This model considers the direct effect of climate on the crop yield. In addition, the alteration of soil fertility due to the same climate change can be considered. Unfortunately, it is still impossible to assess the change in pests and plant diseases depending on the climate change scenarios. The existing mathematical models to assess the impact of climate change on diseases and pests are empirical (CEPAL-FAO, 2013; Donatelli *et al.*, 2017). This means that the parameters of these models are applicable only to the geographic locations where they were assessed and cannot be used in arbitrary other locations without further justification. However, it can be assumed that plant protection practices will be improved in the future to avoid significant crop losses due to plant pests and diseases.

MATERIALS AND METHODS

The study was carried out for four sugar mills in the state of Veracruz: Coatotolapan, San Cristóbal, San Pedro and Tres Valles (Table 1).

The typical climatic conditions for the beginning of the 21st century were calculated using information from the meteorological stations located within the area of each sugar mill (CONAGUA, 2021). While the data on area, soil texture and typical nutrient content for each soil group in the sugarcane plot were obtained from the publications (INEGI, 1988; INEGI, 2004 and SIAP-COLPOS, 2009).

The climatic conditions for the end of the 21st century were assessed based on the climate change predictions for the atmospheric circulation models GFDL-CM3, HADGEM2-ES and MPI-ESM-LR considering two scenarios of change in CO_2 concentration in the

Table 1. Characteristics of the sugar mills in the state of Veracruz where the expected impact of the global climate change on the productivity of rainfed sugarcane has been assessed.

Sugar mills	Municipalities	Cultivated area (ha)	Codes of meteorological stations	Altitude range (masl)	Main soil groups
Coatotolapan	Hueyapan de Ocampo, Acayucan, San Andrés Tuxtla, Juan Rodríguez Clara, Santiago Tuxtla	47,417.25	30035 Cuatotolapan, 30185 Lauchapan, 30184 San Juanillo	10-200	Acrisols, Cambisols, Luvisols, Vertisols
San Cristobal	Carlos A. Carrillo, Cosamaloapan, José Azueta, Chacaltianguis, Ixmatlahuacan, Acula, Tlacojalpan, Amatitlan, Tuxtilla Isla, Tlacotalpan, Otatitlan	123,354.85	30152 Garro, 30117 Paraíso Novillero	0-50	Cambisols, Luvisols, Gleysols
San Pedro	Salta Barranca, Angel R. Cabada, Santiago Tuxtla	16,748.36	30015 Angel R. Cabada, 30143 Tres Zapotes	10-570	Vertisols, Cambisols, Litosoles
Tres Valles	Tres Valles, Cosamaloapan de Carpio, Tierra Blanca, Otatitlan, Tlacojalpan, San Miguel Soyaltepec	55, 104.12	20084 Papaloapan, 30045 Ciudad Aleman	10-50	Cambisols, Luvisols, Vertisols, Gleysols

atmosphere: scenario 4.5 (650 ppm CO_2) and 8.5 (1370 ppm CO_2) for the period 2075-2099 (Cavazos *et al.*, 2013; Fernández *et al.*, 2018).

Rainfed sugarcane (*Saccharum officinarum*) yields have been calculated for the beginning (2005 -2015) and end (2075-2099) of the 21st century with the equation proposed by IIASA/FAO (2012) and FAO (2018):

$$Y_{calc}^{j} = \frac{1}{1-\gamma} F_a^j \sum_i^j \left(Y_{pot}^i K_{hidr}^i \right) \quad (1)$$

where: Y_{calc}^{j} is the agricultural productivity of the commercial raw mass of the crop (kg ha^{-1} year^{-1}), that is, the part of the biomass of the crop produced in the field, registered and used in the mills to produce sugar; Y_{pot}^{i} is the potential productivity of the commercial raw dry mass of sugarcane (kg ha^{-1}) in the absence of water and nutrient restrictions and free of pests or diseases, during month i of year j; K_{hidr}^{j} is the soil moisture coefficient that depends on the intensity of evapotranspiration of the crop and indirectly considers the soil moisture in month i of year j (dimensionless, varies from 0 to 1); F_a^j is the integral soil fertility index; γ is a coefficient that corresponds to the fraction of water content in commercial raw mass of the crop (dimensionless, less than 1).

The yields Y_{calc}^{j} for the beginning of the 21st century were calculated for each of the actual years from 2005 to 2015 and for the end of the century for an average climatic year for which there are climatic predictions at the monthly level, located in the period from 2075 to 2099 (Fernández *et al.*, 2018).

The relative change in sugarcane yield δY_{calc} by the end of the 21st century was determined as follows:

$$\delta Y_{calc} = \frac{Y_{calc}^{2100}}{Y_{calc}^{2000}} = \frac{Y_{pot}^{2100}}{Y_{pot}^{2000}} \frac{K_{hidr}^{2100}}{K_{hidr}^{2000}} \frac{F_{a}^{2100}}{F_{a}^{2000}} = \delta Y_{pot} * \delta K_{hidr} * \delta F_{a} \quad (2)$$

where: $Y_{pot} = \frac{Y_{pot}^{2100}}{Y_{pot}^{2000}}$; $\delta K_{hidr} = \frac{K_{hidr}^{2100}}{K_{hidr}^{2000}}$; $\delta F_{a} = \frac{F_{a}^{2100}}{F_{a}^{2000}}$; Y_{pot}^{2000} and Y_{pot}^{2100} are the potential yields of dry raw matter at the beginning (j=2000) and end (j=2100) of the 21st century; K_{hidr}^{2000} and K_{hidr}^{2100} are the crop water indices in these periods; F_{a}^{2000} and F_{a}^{2100} represent the soil fertility indices for the same period. The values of Y_{pot}^{2000}, Y_{pot}^{2100}, K_{hidr}^{2000}, K_{hidr}^{2100}, F_{a}^{2000} and F_{a}^{2100} are average annual values in the periods studied.

For the beginning of the 21st century, the values of Y_{calc}^{2000}, Y_{pot}^{2000} and K_{hidr}^{2000} were calculated as follows:

$$Y_{calc}^{2000} = \frac{1}{1-\gamma} \frac{\sum_{j=2005}^{2015} Y_{pot}^{j}}{11} * \frac{\sum_{j=2005}^{2015} K_{hidr}^{j}}{11} * F_{a}^{2000} = \frac{1}{1-\gamma} Y_{pot}^{2000} * K_{hidr}^{2000} * F_{a}^{2000} \quad (3)$$

where: $Y_{pot}^{2000} = \frac{\sum_{j=2005}^{2015} Y_{pot}^{j}}{11}$ y $K_{hidr}^{2000} = \frac{\sum_{j=2005}^{2015} K_{hidr}^{j}}{11}$.

The estimation of the potential yields Y_{pot}^{j} has been made with the formula of IIASA/FAO (2012):

$$Y_{pot}^{j} = H * \sum_{i}^{j} \frac{0.36 b_{gm} \beta K_{co_2}}{\frac{1}{N} + 0.25 C_t} \quad (4)$$

where: H is the harvest index or the fraction of the dry biomass of the crop produced in the field which is used to produce sugar (dimensionless, less than one); b_{gm} is the maximum possible intensity of increase in dry biomass (kg ha^{-1} day^{-1}) when the leaf area index *LAI* during the growing season reaches the value 5 (dimensionless); β is the dimensionless relationship between the maximum value of *LAI* in each consecutive month i and the value of LAI=5; K_{co_2} is the coefficient that considers the change in performance in the course of the 21st century with respect to its beginning based on the scenario of growth of the concentration of CO_2 in the atmosphere (dimensionless, greater than or equal to one); N is the duration of the crop season in each consecutive month i or its fraction (days); C_t is a coefficient dependent on the air temperature (day^{-1}).

The values of β and C_t are calculated as follows (IIASA/FAO, 2012):

$$\beta = 0.3424 + 0.9051 \text{ Log}_{10}(LAI) \text{ if } LAI < 5 \text{ and } \beta = 1 \text{ if } LAI \geq 5 \quad (5)$$

$$C_t = 0.0108\left(0.0044 + 0.019T + 0.001T^2\right) \tag{6}$$

where: T is the average air temperature (°C) in each consecutive month i or fraction of the month.

The b_{gm} values are assessed based on the maximum biomass production rate P_m (kg CH_2O ha^{-1} h^{-1}) and some parameters of the photosynthesis of the crop (IIASA/FAO, 2012):

If P_m>20 kg ha^{-1} hour^{-1}

$$b_{gm} = \alpha\left(0.8 + 0.01P_m\right)b_0 + (1-\alpha)\left(0.5 + 0.025P_m\right)b_c \tag{7}$$

If P_m<20 kg ha^{-1} hour^{-1}

$$b_{gm} = \alpha\left(0.5 + 0.025P_m\right)b_0 + (1-\alpha)\left(0.05P_m\right)b_c \tag{8}$$

$$\alpha = \left(A_c - 0.24R_g\right)/\left(0.8A_c\right) \tag{9}$$

where: P_m depends on the air temperature T, type of photosynthesis; b_0 and b_c are intensity of increase in gross dry biomass of the crop in cloudy and clear days, respectively (kg ha^{-1} day^{-1}); A_c is maximum active short-wave radiation on clear days (MJ m^{-2} day^{-1}); R_g is global radiation (MJ m^{-2} day^{-1}).

The values of γ, N, LAI, H, P_m and K_{co_2} for the sugarcane crop were obtained from IIASA/FAO (2012) and Allen *et al.* (2006), the values of A_c, b_0 and b_c —from Driessen and Konijn (1992).

The water coefficient K^j_{hidr} was determined as follows (IIASA/FAO, 2012; FAO, 2018):

$$K^j_{hidr} = 1 - k^y\left(1 - \frac{ET^{pl}_a}{ET^{pl}_0}\right) \tag{10}$$

where: k^y is the coefficient of sensitivity of the sugarcane crop to the deficiency of water in the soil (FAO, 2012); ET^{pl}_a refers to the actual intensity of crop evapotranspiration when soil moisture is equal to θ; ET^{pl}_0 indicates the intensity of potential evapotranspiration in case of water deficiency in the soil.

The typical change in the value of $\left(ET^{pl}_a / ET^{pl}_0\right)$ as a function of soil moisture θ in the active root zone is expressed as follows:

$$\frac{ET^{pl}_a}{ET^{pl}_0} = \frac{\theta - PM}{CC - PM},\ \text{when } PM \le \theta \le CC \text{ and } \frac{ET^{pl}_a}{ET^{pl}_0} = 1,\ \text{when } \theta \ge CC \tag{11}$$

where: θ - volumetric soil moisture content in the active root zone; *CC* and *PM* are the soil moisture values corresponding to field capacity and wilting point, respectively.

The determination of the K_{hidr}^{j} coefficient was carried out using the CROPWAT program (FAO, 2009).

In order to obtain the fertility index of agricultural F_a^j en sugarcane plots, the equation proposed by Pegov and Jomyakov (1991) was used:

$$F_a^j = 0.46\,\frac{OM}{OM_{max.}} + 0.28\sqrt{\frac{P}{P_{max.}}\frac{K}{K_{max.}}} + 0.26\,e^{-\left(\frac{pH-6}{2}\right)^2} \qquad (12)$$

where: F_a^j is the fertility index at the beginning (j=2000) and end (j=2100) of the 21st century. *OM*, *P*, *K* and *pH* are the modal values of organic matter, phosphorus and potassium content available for the crop and pH, respectively, in the 0-20 cm soil layer of the sugarcane plots at the beginning. (j=2000) and end (j=2100) of the 21st century. The F_a value is dimensionless and varies between 0 and 1, where 0 corresponds to a completely degraded or infertile soil and 1 to a soil with maximum fertility.

In order to calculate the sugarcane yields, the monthly climatic data at the beginning of the 21st century during the period 2005-2015 and the soil fertility factors mentioned in formula (12) have been analyzed. The calculated yields were compared with the productivity reported in publications (Aguilar-Rivera, 2014; Flores-Granados, 2017; SIAP, 2022).

The annual yields calculated for the period from 2005 to 2015 $\left(Y_{calc}^{2000}\right)$ for each of the sugar mills were compared with the observed yields $\left(Y_{obs}^{2000}\right)$. This comparison was realized between the normalized yields $Y_{calc}^{2000}/Y_{calc\,max}^{2000}$ and $Y_{obs}^{2000}/Y_{obs\,max}^{2000}$, where $Y_{calc\,max}^{2000}$ and $Y_{obs\,max}^{2000}$ are the maximum yields calculated and observed during the period from 2005 to 2015 in each sugar mill.

The objective of the comparison was to improve the fit between the calculated and observed crop yields, minimizing possible errors due to ignorance of some factors that influence the observed data, such as the possible mass loss of the harvested sugarcane during the burning process or transportation, or due to pests, diseases, etc. The parameters of the regression equation were established between the values $Y_{calc}^{2000}/Y_{calc\,max}^{2000}$ and $Y_{obs}^{2000}/Y_{obs\,max}^{2000}$ for each of the sugar mills.

Then, taking into account the parameters of the regression equation, the yields for the end of the 21st century $\left(Y_{calc}^{2100}\right)$ were calculated and the δY_{calc} values were estimated according to formula (2).

The alteration of the fertility of agricultural soils at the end of the 21st century due to the climate change was assessed using the methodology developed by Nikol'skii *et al.*

(2006) and Castillo *et al.* (2007). This methodology is based on the use of the quantitative relationship between the regional modal values of the integral fertility index of a virgin soil not used in agriculture F_{vrg}^{j} and a radiative index of dryness I_{vrg}^{j} regional mean annual established for the beginning of the 21st century, that is, for *j*=2000, for relatively flat terrain with surface slopes less than 3% in different climatic zones of Mexico (Nikol'skii *et al.*, 2006; Castillo *et al.*, 2007).

The climatic index I_{vgn}^{j} for virgin soils not used in agriculture is calculated as follows (Budyko, 1977; Koster and Suarez, 1999):

$$I_{vgn}^{j} = \frac{Rn_{vrg}^{j}}{\lambda Pr^{j}} \qquad (13)$$

where: Rn_{vrg}^{j} and Pr^{j} are the mean annual measured values of net radiation (KJ m^{-2}) and precipitation (mm) typical for the virgin soils in the region of sugarcane production at the beginning (*j*=2000) and end (*j*=2100) of the XXI century; λ is the latent heat of evaporation equal to 2.51 KJ m^{-2} mm^{-1}.

In order to calculate the climatic index for the agricultural sugarcane lands I_{a}^{j} at the beginning and end of the 21st century, the mean annual value of net radiation Rn_{a}^{j} was determined using the following formula (Allen *et al.*, 2006):

$$Rn_{a}^{j} = (1-\alpha) Rg_{a}^{j} - Rb_{a}^{j} \qquad (14)$$

where: α is the albedo of the sugarcane plot (α=0.15); Rg_{a}^{j} and Rb_{a}^{j} are global radiation (incoming radiation) and outgoing longwave radiation, respectively, in the year *j* (MJ m^{-2} day^{-1}). The Rg_{a}^{2000} and Rb_{a}^{2000} values for agricultural lands with sugarcane at the beginning of the 21st century were calculated with climatological data (CONAGUA, 2021); while the values of Rg_{a}^{2100} and Rb_{a}^{2100} were obtained from the publications by Cavazos *et al.* (2013) and Fernández *et al.* (2018).

The previously established graphs on changes in regional values of the integral fertility index of virgin soils F_{vrg}^{2000} and its components at the beginning of the 21st century depending on the climate index I_{vrg}^{2000} were used to assess the impact of the climate change on agricultural soil fertility in sugarcane lands $\left(F_{a}^{2100} / F_{a}^{2000}\right)$ at the end of 21st century. An example of such graphs is shown in Figure 1 (Nikol'skii *et al.*, 2010).

The methodology for obtaining similar graphs and their application to predict changes in some soil properties due to climate change was described in detail in several publications (Nikol'skii *et al.*, 2006; Castillo *et al.*, 2007; Terrazas-Mendoza *et al.*, 2010).

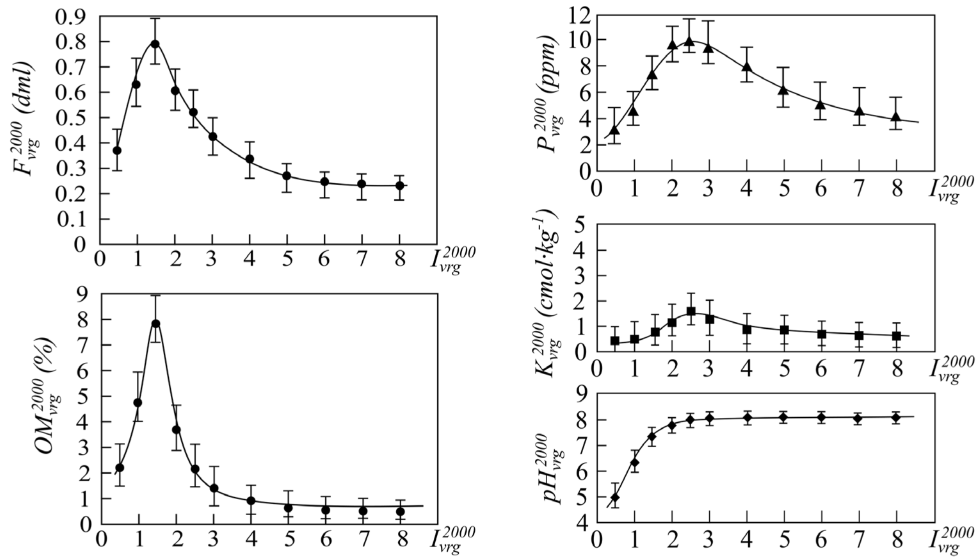

Figure 1. Dependence of the modal values of the integral virgin soil fertility index F_{vrg}^{2000} and its components MO_{vrg}^{2000}, P_{vrg}^{2000}, K_{vrg}^{2000} and pH_{vrg}^{2000} according to formula (12) at the beginning of the 21st century based on the climatic index I_{vrg}^{2000} typical for the land with slopes less than 3% and soils deeper than 1 m. Confidence intervals are shown. (Nikol'skii *et al.*, 2010).

In accordance with the Geographical Law of Soil Zonality and considering a relatively slow climate change at a mean annual level, the $F_{vrg}^{2000}\left(I_{vrg}^{2000}\right)$ relationship established for the beginning of the 21st century should be preserved at the end of the same century. Therefore, by knowing how the I_a^j index on agricultural land will change at the end of the century compared to its beginning, one can estimate the change of the agricultural soil fertility index due to climate change alone. For this, it is necessary to take the values of F_{vrg}^{2000} and F_{vrg}^{2100} from the relationship $F_{vrg}^{2000}\left(I_{vrg}^{2000}\right)$ corresponding to the climatic index of the sugarcane land I_a^{2000} and I_a^{2100} for the beginning and end of the 21st century. The regional ratio $F_{vrg}^{2100}/F_{vrg}^{2000}$ can be applied to the local ratio F_a^{2100}/F_a^{2000} for sugarcane lands as follows:

$$F_a^{2100} = F_a^{2000}\left(F_{vrg}^{2100}/F_{vrg}^{2000}\right) \qquad (15)$$

where: F_a^{2000} and F_a^{2100} are the mean annual values of the integral soil fertility index of the sugarcane lands at the beginning and end of the 21st century, respectively. The F_a^{2000} values are determined with formula (12). F_{vrg}^{2000} and F_{vrg}^{2100} are the regional annual average values of the integral fertility index corresponding to virgin soils of the same region obtained from Figure 1 based on the known values of the climatic index I_a^{2000} and I_a^{2100}.

RESULTS AND DISCUSSION

Figure 2 shows the observed sugarcane yields $\left(Y_{obs}^{2000}\right)$ at the beginning of the 21st century in each of the selected sugar mills compared to the calculated yields $\left(Y_{calc}^{2000}\right)$. The observed yield data have been obtained from publications by Aguilar-Rivera (2014), Flores-Granados (2017) and SIAP (2022). The yields are presented in normalized form $\left(Y_{calc}^{2000} / Y_{calc\,max}^{2000}\right)$ and $\left(Y_{obs}^{2000} / Y_{obs\,max}^{2000}\right)$, where $Y_{calc\,max}^{2000}$ and $Y_{obs\,max}^{2000}$ are the maximum yields calculated and observed during the period from 2005 to 2015 in each sugar mill. The values of Y_{calc}^{2000}, $Y_{calc\,max}^{2000}$, Y_{obs}^{2000} and $Y_{obs\,max}^{2000}$ correspond to the commercial raw mass of the crop.

The figure shows two lines of adjustment as well. Continuous line is theoretical as a reference which corresponds to the case of complete coincidence between the calculated and observed yields: $Y_{calc}^{2000} / Y_{calc\,max}^{2000} = Y_{obs}^{2000} / Y_{obs\,max}^{2000}$. Dashed line is real and corresponds to the different regression equation: $Y_{calc}^{2000} / Y_{calc\,max}^{2000} = a\left(Y_{obs}^{2000} / Y_{obs\,max}^{2000}\right) + b$, where a and b are constants.

The regression equations of the calculated and observed yields are presented in the Table 2.

It was found that the linear relationship between the calculated maximum yield and the maximum real yield obtained with the fitted equations is statistically significant with a confidence level of 95%. In addition, a good fit is observed in all the obtained equations,

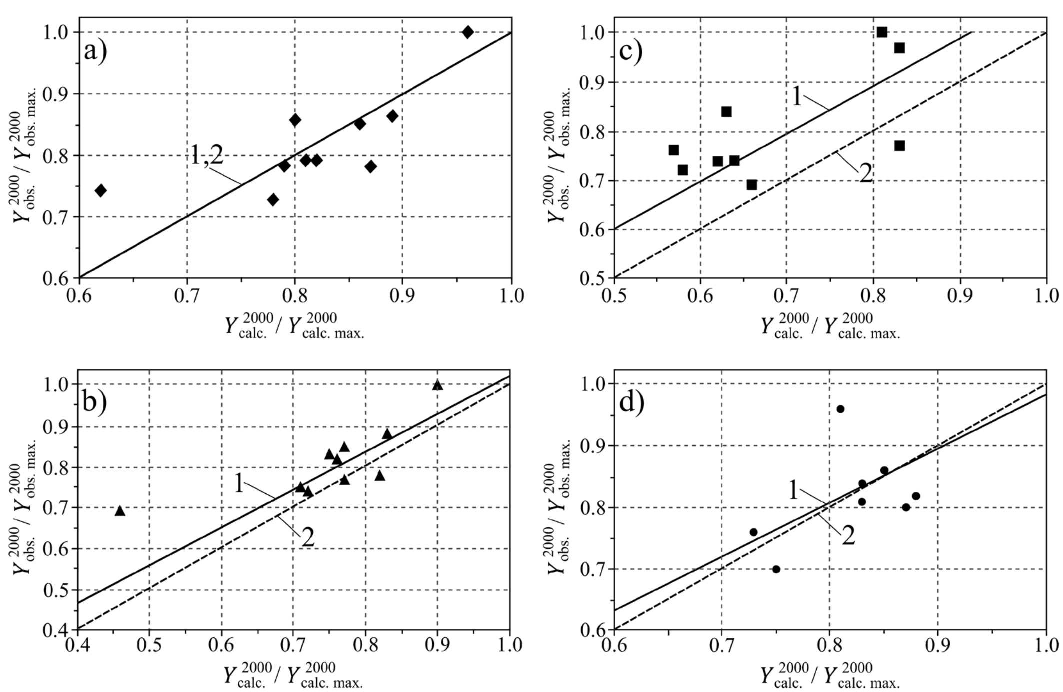

Figure 2. Graphs between the sugarcane yields calculated $\left(Y_{calc}^{2000} / Y_{calc\,max}^{2000}\right)$ and observed $\left(Y_{obs}^{2000} / Y_{obs\,max}^{2000}\right)$ in the sugar mills of the state of Veracruz at the beginning of the 21st century (a - Coatotolapan; b - San Cristobal; c - San Pedro; d - Tres Valles). 1 – theoretical adjustment; 2 – actual setting.

Table 2. Adjustment regression equations of calculated and observed sugarcane yields.

Sugar mill	Regression equation	R^2	$\sigma_{\bar{x}}$	$Y_{obs\,max}$ (t ha^{-1})	$Y_{calc\,max}$ (t ha^{-1})
Coatotolapan	$\frac{Y_{obs}^{2000}}{Y_{obs\,max}^{2000}}=0.95\frac{Y_{calc}^{2000}}{Y_{calc\,max}^{2000}}+0.04$	0.98	0.06	54.13	56.24
San Cristóbal	$\frac{Y_{obs}^{2000}}{Y_{obs\,max}^{2000}}=0.93\frac{Y_{calc}^{2000}}{Y_{calc\,max}^{2000}}+0.1$	0.95	0.08	56.26	58.68
San Pedro	$\frac{Y_{obs}^{2000}}{Y_{obs\,max}^{2000}}=0.95\frac{Y_{calc}^{2000}}{Y_{calc\,max}^{2000}}+0.12$	0.93	0.10	66.46	67.11
Tres Valles	$\frac{Y_{obs}^{2000}}{Y_{obs\,max}^{2000}}=0.85\frac{Y_{calc}^{2000}}{Y_{calc\,max}^{2000}}+0.13$	0.98	0.06	58.40	60.75

since in all cases we have a correlation coefficient greater than 0.9, the equation obtained for the San Pedro mill being the one with the lowest correlation. The difference in the quality of the fit possibly could be explained by the difference in the quality of the initial climatic and edaphic data.

Finally, Table 3 presents the results of the assessed impact of climate change at the end of the 21st century on the productivity of sugarcane in the state of Veracruz. This table estimates expected changes in relative yield $\delta Y_{calc}=Y_{calc}^{2100}/Y_{calc}^{2000}$ and its components δY_{pot}, δK_{hidr} y δF_a calculated with equation (2).

Analyzing the data in Table 3, it can be concluded that the three atmospheric circulation models (GFDL CM3, HADGEM2 and MPI ESM LR) recommended by Cavazos *et al.* (2013) show similar results of variation in change of sugarcane yield (δY_{calc}), although there is a small difference between its components (δY_{calc}).

The assessment of the impact of climate change on sugarcane indicates that in the case of a doubling of the concentration of CO_2 in the atmosphere at the end of the 21st century compared to the current one, the yields of the crop will be lower than the current ones of 8 to 15% in the sugar mills of Cuatotolapan, San Cristóbal and San Pedro. If the CO_2 concentration increases four times, the yields will be lower than the current ones of 11 to 20%. For the Tres Valles sugar mill, a conservation of the current yield is expected.

The expected increase in air temperature, photosynthetically active radiation and CO_2 concentration in the atmosphere will lead to a change in potential productivity (δY_{pot}) from a decrease of 12% to an increase of 13%, depending on location of sugar mill, model and scenario of CO_2 growth in the atmosphere. But most of the values of $\delta Y_{pot}=\delta Y_{pot}^{2100}/\delta Y_{pot}^{2000}$ are in the interval 0.95 to 1.05, which means that in both CO_2 growth scenarios, potential productivity will be practically unchanged.

It was found that the main factor for the decrease in the sugarcane productivity is a decrease of 4 to 22% of the soil moisture coefficient K_{hidr}^{j} mainly due to the decrease

Table 3. Change in the yield of sugarcane (*Saccharum officinarum*) (δY_{calc}), and its components (δY_{pot}, δK_{hidr} y δF_a) assessed with equations (1) and (2) in four sugar mills in the state of Veracruz for the end of the 21[st] century (2075-2099) corresponding to the GFDL CM3, HADGEM2 and MPI ESM LR climate models with two scenarios of the CO_2 concentration in the atmosphere growth (RCP): 4.5 (650 ppm CO_2) and 8.5 (1370 ppm CO_2).

Ingenio	Modelo	RCP 4.5				RCP 8.5			
		δY_{pot}	δK_{hidr}	δF_a	δY_{calc}	δY_{pot}	δK_{hidr}	δF_a	δY_{calc}
Coatotolapan	GFDL CM3	0.88	0.91	1.08	0.85	0.90	0.96	0.98	0.82
	HADGEM2	1.02	0.84	1.02	0.85	0.82	0.99	1.01	0.80
	MPI ESM LR	1.00	0.93	0.96	0.86	0.95	0.93	0.96	0.82
San Cristóbal	GFDL CM3	0.94	0.87	1.09	0.89	0.95	0.87	1.07	0.86
	HADGEM2	1.04	0.84	1.03	0.89	0.89	0.85	1.12	0.84
	MPI ESM LR	0.98	0.91	1.00	0.91	0.90	0.92	1.07	0.87
San Pedro	GFDL CM3	1.03	0.80	1.09	0.90	1.07	0.82	1.02	0.89
	HADGEM2	1.06	0.78	1.10	0.91	1.04	0.81	1.03	0.87
	MPI ESM LR	1.13	0.80	1.02	0.92	1.04	0.82	1.03	0.88
Tres Valles	GFDL CM3	1.01	0.96	1.04	1.01	0.93	0.98	1.08	0.99
	HADGEM2	1.05	0.89	1.08	1.01	1.11	0.86	1.04	0.99
	MPI ESM LR	0.97	0.96	1.08	1.01	1.06	0.90	1.04	1.00

in precipitation during the relatively dry season (from November to May) which causes a decrease in soil water reserves in the root zone during the crop cycle. The effect of an increase in air temperatures and solar radiation on an increase in evapotranspiration and a decrease in the K_{hidr}^{j} coefficient is insignificant.

Regarding the alteration of soil fertility due to climate change, no large changes are expected in the integral fertility index F_a in both scenarios of the growth of CO_2 concentration in the atmosphere. The typical variation of the value of $\delta F_a = F_a^{2100} / F_a^{2100}$ in the sugarcane lands is within the range of 1.00 to 1.05, except for the San Cristóbal mill, where δF_a varies in a slightly wider range, from 0.96 to 1.09 in the case of doubling of the CO_2 concentration and from 1.07 to 1.12 in case of four-fold growth of CO_2 concentration.

The results on the expected change in sugarcane productivity at the end of the 21[st] century is somewhat consistent with the results obtained by Marin *et al.* (2013). Although these authors use a different approach to the present one and conclude that sugarcane productivity will be increased insignificantly, they point out that this productivity may be limited by CO_2, temperature and solar radiation.

According to the information obtained in this study, we can point out that the main factor in the expected decrease in sugarcane productivity is due to the decrease in precipitation during the season with less precipitation (from November to May). It should be noted that the comments on the expected impact of climate change on the sugarcane productivity considers the case of application in future of current plant varieties and technologies of crop, soil and water management, although it should considered an improvement of crop varieties and the agricultural technology.

CONCLUSIONS

The assessment of impact of the climate change on the sugarcane productivity for four sugar mills in the state of Veracruz, carried out using the physiological model of crop development, proposed by IISA/FAO, indicates that at the end of the 21st century there is a risk of loss of sugarcane production up to 20%, mainly due to the decrease in precipitation.

Sugarcane crop yield calculations for the beginning of the 21st century indicate that there is a rather good coincidence between calculated and observed crop yields. The correlation coefficients between the calculated and observed yields are in a range of 0.93-0.98 with standard error between 0.06-0.10. This means that the IISA/FAO physiological model based on agroecological principles, and a limited number of climatic and edaphic variables can be used as a tool to estimate the impact of climate change on sugarcane productivity.

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Colegio de Postgraduados

Effect of the elicitation with magnetic field of corn seeds on the development and nutrition of sprouts

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ABSTRACT

Objective: To evaluate the effect of exposing corn seeds to a 100 mT magnetic field (MF) on their sprout development.

Design/methodology/approach: A completely randomized design was used, with five treatments (0, 10, 15, 30, and 60 minutes of MF exposure) with three repetitions (72 experimental units). From the germination process, the gibberellic acid concentration (GA_3) and α-amylase activity were determined, morphometric and biochemical parameters of the foliar tissue from the sprouts were measured, such as total phenols, flavonoids, and catalase (CAT), peroxidase (POX) and phenylalanium ammonium lyase (PAL) activities. Finally, some nutritional quality parameters of the sprouts were quantified, such as protein and ash content.

Results: The results showed that the treatment of corn seeds with a MF had a favorable effect on the germination process increasing the GA_3 concentration. Also, improvement in the development and quality of the sprouts, by increasing the growth of the shoot, root length, concentration of phenolic compounds and ash content in corn sprouts was found.

Limitations on study/implications: escalate elicitation to the field level.

Findings/conclusions: The elicitation of corn seeds with a magnetic field generates positive changes that transcend the corn sprouts.

Keywords: physical biostimulation of seeds, corn sprouts, *Zea mays*.

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INTRODUCTION

The nutritional quality of plants is a crucial factor to ensure their optimal growth and ability to respond to different environmental conditions. In this sense, applying innovative techniques that promote the improvement of the nutritional quality of plants, from their initial developmental stages, such as germination, has been the subject of growing interest in agricultural research (Bai *et al.*, 2019). Corn (*Zea mays* L.) is one of the most important crops worldwide, due to its extensive utilization in human and animal nutrition (Pingali, 2012). However, the nutritional content of corn seeds is affected by various factors, such as their growing conditions and nutrient availability in the soil. In this sense, researchers

explore new strategies that improve the nutritional quality of corn plants from their initial phase. Magnetic field (MF) exposure on seeds has been shown to have positive effects on different aspects of plant development, including germination, growth, and response to biotic and abiotic stress (Davies and Abeles, 1991). In this context, this research aims to investigate the MF effects on the germination process and changes in the development and nutritional quality of corn sprouts.

MATERIALS AND METHODS

Each treatment was done, in triplicate, with 72 C.V. napalú corn seeds as an experimental unit, exposed to a MF of 100 mT for 10, 15, 30, and 60 minutes. The research was divided into two stages, on the germination process at 72 hours and 7-day sprouts.

Within the germination process, the gibberellic acid (GA_3) concentration was quantified following the Holbrook *et al.* method (1981), and the α-amylases activity following the Miller *et al.* method (1959).

In the sprouts, shoot height and root length were measured. The assessed biochemical and nutritional parameters of sprout foliar tissue were total phenols (Folin-Ciocalteu), flavonoids (aluminum trichloride method), phenylalanine ammonium lyase (PAL) activity, by Beaudoin-Eagan (1985); catalases (CAT) and peroxidases (POX) activity described by Hanna *et al.* (2011). Finally, the protein and ash content were determined following the Bradford method and by Colombato (2000) respectively. A statistical analysis (simple ANOVA) was performed on the results obtained from each experiment, using the Statgraphics Centurion XVIII statistical software, using the Tukey's mean comparison test ($P<0.05$).

RESULTS AND DISCUSSION

The results indicated that the MF treatment on corn seeds had a favorable effect on the germination process, increasing the GA_3 concentration (Table 1).

The elicitation of corn seeds to MF generated a significant increase in the GA_3 concentration, from 15 minutes of exposure. This phytohormone plays an important role in seed germination and stem elongation (Salisbury and Ross, 1992). Similar results were reported by Podleśny *et al.* (2021), where pea seeds exposure to a MF caused a GA_3 increased content. This may be because MF not only affects the chemical properties

Table 1. Effect of corn seeds elicitation with a MF on the gibberellic acid concentration and the enzymatic activity of α-amylases involved in the germination process.

Treatments	**GA_3 (μg g^{-1} fresh weight)**	**α-amylases (U mg^{-1} protein)**
Control	1329.89±68.42 c	0.083±0.00 a
CM-10	1356.56±68.42 c	0.123±0.01 a
CM-15	1889.89±93.65 a	0.110±0.02 a
CM-30	1707.67±66.66 ab	0.086±0.00 a
CM-60	1627.67±46.18 b	0.085±0.02 a
DMS (P<0.05)	127.897	0.032

of plants but also generates various physical changes in the properties of solutes within the plant cell (Galland *et al.*, 2005). This research demonstrated that there were no changes in the α-amylases activity during the germination of maize seeds previously exposed to MF.

The elicitation of corn seeds through MF not only changes the germination process but also induces changes in the development and growth of corn sprouts (Table 2).

Elicitation of seeds with CM for 60 minutes caused a significant increase of 30% in shoot height and a 21% increase in root length concerning the control. The improvement in root length is a positive result since it would allow the seedling to extract moisture and absorb more nutrients from the soil and, as a consequence, achieve greater growth in the seedlings (Saktheeswari and Subrahmanyam, 1989). The secondary metabolism of sprouts due to exposure of corn seeds to CM increased (Table 3).

The seed's exposure to MF significantly increased the total phenols and flavonoids concentration in the foliar tissue of the sprouts, the 15-minute treatment being the one that had the greatest increase; this concentration relates to the PAL activity (Wei *et al.*, 2015). The results of the antioxidant enzyme activity are depicted in Table 4. The 30-minute exposure of corn seeds significantly increased the CAT activity compared to the control. POX activity had no significant differences. Cheng and Zhang (2016) report contrary results, where the exposure of corn seeds to 200 mT MF in a 3 h exposure period increased CAT activity by 34% so the intensity and exposure time are important factors to consider.

Table 2. Measurement of morphometric parameters of corn sprouts obtained from magnetic field seed elicitation.

Treatments	Shoot height (mm)	Root length (mm)
Control	62.45±17.53 b	20.01±6.31 b
CM-10	51.79±18.90 c	16.70±6.94 c
CM-15	63.59±19.20 b	22.01±6.50 b
CM-30	71.01±16.20 b	22.89±5.55 ab
CM-60	81.87±20.15 a	24.56±8.42 a
DMS ($P<0.05$)	6.15	2.23

Table 3. Total phenols, flavonoids, and enzymatic activity of PAL in leaf tissue of sprouts obtained from the elicitation of corn seeds by magnetic field.

Treatments	Total phenols (mg g^{-1} dry weight)	Flavonoids (μg g^{-1} dry weight)	PAL activity (U mg^{-1} of protein)
Control	1.05±0.02 c	7.20±0.43 c	0.039±0.007 b
CM-10	1.37±0.14 b	10.70±0.33 b	0.047±0.004 b
CM-15	1.68±0.03 a	12.53±0.47 a	0.076±0.016 a
CM-30	1.39±0.14 b	10.79±0.74 b	0.049±0.011 ab
CM-60	1.55±0.01 ab	10.56±0.41 b	0.057±0.009 ab
DMS ($P<0.05$)	0.17	0.90	0.019

Within the assessed nutritional parameters (Table 5), we can highlight that the 15-minute exposure of corn seeds to MF doubled the ash content compared to the control. While for the protein content, there were no significant differences compared to the control, this is not a negative result since the protein content is maintained and not affected by the interaction of the magnetic field with the corn seeds.

There is no scientific evidence that describes the effect of MF on the ash content increase during plant germination. However, some studies suggest that MF can improve nutrient absorption and mineral accumulation in plants, which could have an indirect effect on the ash percentage in the sprouts. For example, Zhang *et al.* (2014) found a Fe accumulation in the roots of white corn plants. Guo and collaborators (2016) observed a Ca, Mg, and Fe accumulation in the roots of tomato plants. Also, Jafari, Ahmadi & Fathi (2018) found greater nitrogen (N), phosphorus (P), and potassium (K) uptake in wheat plants through nutrient uptake by plant roots.

Various hypotheses have been proposed about how magnetic fields improve nutrient absorption and mineral accumulation in plants. One of them is that the magnetic fields affect the plant root cell membrane structure, which in turn improves membrane permeability and nutrient absorption (Dhiman & Sastry, 2014).

Table 4. Effect of corn seeds elicitation with a magnetic field on the antioxidant enzymes activity of sprout foliar tissue.

Treatments	CAT activity (U mg^{-1} of protein)	POX activity (U mg^{-1} of protein)
Control	122827.66±4788.2 b	1.760±0.32 a
CM-10	103444±2660.94 d	1.402±0.38 a
CM-15	114859±298.264 c	1.389±0.47 a
CM-30	167339±1578.67 a	1.160±0.53 a
CM-60	78788.7±1662.61 e	0.951±0.17 a
DMS (P<0.05)	4837.55	0.728

Table 5. Protein and ash content of corn sprouts obtained from seeds elicitated with a magnetic field.

Treatments	Protein ($mg\ g^{-1}$ fresh weight)	Ashes (%)
Control	0.015±0.0004 a	2.0±0 b
CM-10	0.015±0.0003 a	3.0±1 ab
CM-15	0.016±0.0003 a	4.0±0 a
CM-30	0.015±0.0001 a	3.0±0 ab
CM-60	0.016±0.0002 a	2.6±0.5 ab
DMS (P<0.05)	0.001	0.939

CONCLUSIONS

The elicitation of corn seeds with a magnetic field is an alternative to improve the development and quality of sprouts, by increasing the growth, phenolic compounds, and ash content in corn sprouts.

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Colegio de Postgraduados

Control of the whitefly *Bemisia tabaci* (Genn.) in greenhouses

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ABSTRACT
A formulation based on neem oil (*Azadirachta indica*), chamomile (*Matricaria chamomilla*), lechuguilla (*Agave lechuguilla* Torrey) and cactus pectin extracts at different concentrations was used in greenhouses to control the whitefly *Bemisia tabaci* (Genn.). For moth monitoring, ecological water traps were used to which Noctovi® dipteran pheromones and a food component based on 50% sugar and 20% powdered yeast were added. The work was carried out in six tomato and strawberry production greenhouses located in the city of Durango, in northern Mexico, from August to November 2014. Plant extracts formulated with lechugilla surfactant based on chicalote (*Argemone mexicana* L.), skunk epazote (*Chenopodium glaucum*) and higuerilla (*Ricinus communis*) were used as controls and sprayed at intervals of 15 days each. Field results indicate that the formulation based on neem oil, nopal pectin, chamomile and lechuguilla extracts presented a moth mortality rate of 60% in tomato crops and 62% in strawberry crops.

Keywords: Neem oil, greenhouse, whitefly.

INTRODUCTION

Tomato (*Lycopersicum solanum*) is among the most economically important crops worldwide, with China and the USA being the main producers in the world, yielding more than 198 million tons (López and Estrada, 2005). Mexico is the main international exporter, sending the product to the USA, Canada, and El Salvador; in 2011 alone, 1,872,000 tons were produced (PRODUCE, 2012). In the state of Durango, Mexico, there is an approximate area of 150 hectares of greenhouse structures, 268 hectares of shade netting and 23 hectares of shade house (SIAP, 2014), within these areas, horticultural products such as: pumpkin, onion, strawberry, bell pepper, tomato and broadleaf vegetables are grown. These crops can be attacked by insects such as whiteflies, aphids, thrips and paratrioza, which are vectors of virus, bacterial and fungal diseases that cause, among other ailments, rotting of the stem, root, and fruit, reducing the quality and quantity of production by up to 60% (García and González, 2006). *Bemisia tabaci* (Geen.) has a reproductive cycle consisting of egg, four nymphal stages, pupa, and adult, which lasts approximately 28 days at average temperatures of 20-22 °C. Adults and nymphs are located on the underside of leaves, especially in the apical buds. The whitefly (Hemiptera: Aleyrodidae) is a phytophagous insect that causes damage to plants; as an imago, it feeds on the phloem by sucking the assimilated photos with its stylet (Rosell *et al.*, 1995). Greenhouses possess temperature and relative humidity conditions for the optimal growth of pests such as the whitefly and other diseases (Monroy, 2010).

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Among the organic alternatives to counteract the harmful effect caused by whiteflies are neem extracts (*Azadirachta indica* Juss.). This biorational has given good results, exhibiting several advantages such as high biodegradability and low risk to human health (Lannacone and Lamas, 2002). A pest control method used by producers is to use systemic action insecticides, based on organochlorine, organophosphorus and pyrethroid compounds; on average 2.0 liters per hectare are applied, a situation that is causing environmental and health deterioration to the final consumers due to the residual nature of the product in the plant tissue. The objective of this work is the biological control of the whitefly, *Bemisia tabaci* (Genn.) in protected environments through bioformulates with the use of biorational extracts of neem, chicalote, skunk epazote, higuerilla and chamomile at different doses and concentrations.

MATERIALS AND METHODS

Field work was performed from August to December 2014, in six greenhouses with a size of 10 meters wide by 200 meters long where vegetables, mainly tomato and strawberry (Table 1) are produced. Additionally, bioassays were conducted in a CR® brand bioclimatic chamber with controlled temperature and humidity. T1: bioformulate based on neem extract (*Azadirachta indica*), chamomile (*Matricaria chamomilla* L.), nopal pectin and lechuguilla extract (*Agave lechuguilla* Torrey) in a proportion of 25, 50, 15, 10%. T2: based on skunk epazote extract (*Dysphania graveolens*) and lechuguilla extract at a ratio of 70, 30%. T3: based on extract of chicalote (*Argemone mexicana*) and lechuguilla extract at a ratio of 70, 30%. T4: based on higuerilla (*Ricinus communis*) seed extract and lechuguilla extract at a ratio of 70, 30%. Three replicates were carried for each treatment under a completely randomized design for a total of 12 experiments, when significant differences were detected in the analysis of variance at a $p<0.05$ a comparison of means was performed by DMS using the Olivares, 1994 statistical software. In each experiment, 50 adult whitefly specimens were placed in a plastic container measuring 8×15 cm, bioformulates were

Table 1. Location of the coopertaing greenhouses.

Greenhouse	Location
Santa Rosa	Km 22 México Highway. 24.013459 N, −104.436055 W
Santa Cruz	Km 52 Nombre de Dios-Poanas Highway Durango. 23.907906 N, −104.132659 W.
Municipales	Eastern wastewater treatment plant PTAR. 24.026888 N, −104.602761W.
Diez de Santa Lucia	Km 2 Málaga Highway Durango. 24.133390 N, −104.543595 W.
Gabino Santillán	Gabino Santillán town Durango. 23.982207 N, −104.573153 W.
Diego Berlanga	Gabino Santillán town Durango. 23.982207 N, −104.573153 W.
Vermi Orgánicos de Durango	Parral km 2.5 Highway Durango. 24.099605 N, −104.696247 W.

sprayed twice a day, percentage of mortality was then evaluated. The bioformulation was developed in five stages:

First: extraction of cactus pectin, which is used to obtain an organic emulsifier in a proportion of 20 grams in 100 milliliters of water, until boiling for 10 minutes. The technique consists of: using fresh nopal of any variety, unstemmed and without thorns, preferably cut the same day or refrigerated to avoid desiccation and loss of volatile compounds, for every kilogram of fresh nopal, approximately 20 grams of filtered, purified nopal pectin are obtained, the objective of using this pectin is to emulsify the neem oil and make it soluble in water. For the formulation of the chicalote, epazote de zorrillo and higuerilla extracts, 100 grams of fresh leaves were placed separately in two liters of water, the surfactant based on lechuguilla was added in a mixture of 30% each in liquid form, then placed in 5.0 liter containers and stored under refrigeration.

Second: obtaining the surfactant, the process consists of using the leaves of lechuguilla (*Agave lechuguilla* Torrey), which is defibrated and using the residue of the carving called guishe, which contains up to 50% surfactant.

Third: obtaining chamomile extracts, the process consists of boiling the chamomile with six sachets per 1.0 liter of water for 10 minutes, strain and let cool.

Fourth: Use of commercial organic neem oil in 1.0 liter presentation.

Fifth: Elaboration of the trap, which consists of a 15×20 cm sheet of blue color for diptera and yellow color for lepidoptera with the following characteristics: suitable with a solar led bulb that works for 8 hours at night and is charged with light during the day. An open container with a mixture of Noctovi® pheromones and food composed of 50% sugar, 20% yeast and water.

RESULTS AND DISCUSSION

After different laboratory tests to achieve an effective emulsification and proven efficiency in terms of mortality in greenhouses, the mixture employed for this study was composed as follows: 40% *Agave lechuguilla* extract, 10% neem oil, 10% emulsifier (cactus pectin) and 10% chamomile extract, 5 ml per plant without direct dilution were applied. The results obtained for the control of whitefly, aphids, and thrips in two greenhouses employing this mixture are shown in Figure 1, where it can be observed that the percentage of whitefly mortality was 60% in the tomato crop in the Gabino Santillan greenhouse and 62% in the strawberry crop in the Vermi Organicos greenhouse, respectively. As for thrips (*Frankliniella occidentalis*). The formulated product reduced the presence of this pest by 15% in the tomato crop and by 10% in the strawberry crop. The results regarding the analysis of variance for each of the treatments used in a bioclimatic chamber with a controlled environment are shown in Table 2.

The ANVA results show that there is statistical significance among the treatments (formulations), the comparison of means is shown in Table 3.

Table 3 shows that the formulation based on neem oil, with cactus pectin, chamomile and lechuguilla agave extract, exhibited the best efficiency in whitefly mortality with 71.3%, 6% more compared with the other three formulations that statistically presented the

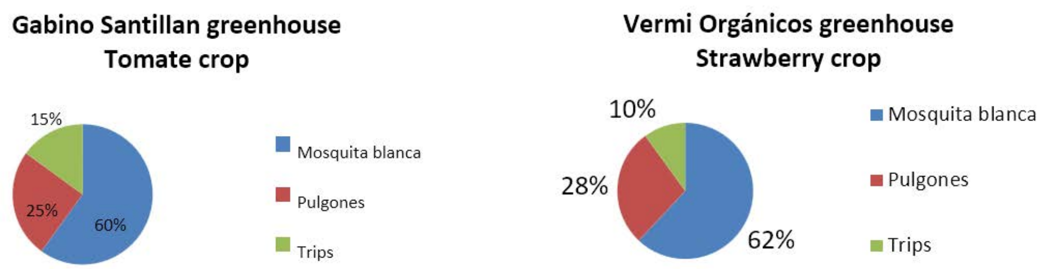

Figure 1. Results obtained in pest control, using a formulation based on neem oil, nopal pectin, chamomile extract and lechuguilla extract.

Table 2. ANVA of the bioclimatic chamber bioassays.

FV	GL	SC	CM	F	P>F
Treatments	3	189.582031	63.194012	11.1512	0.004
Error	8	45.335938	5.666992		
Total	11	234.917969			

P<0.05

Table 3. Comparison of means obtained from bioassays with *Bemisia tabaci* in bioclimatic chamber.

Formulate	Mortality (%)
Neem oil, chamomille extract, cactus pectin, lechuguilla extract	71.3 a
Epazote de zorrillo	65.0 b
Chicalote	62.3 b
Higuerilla	61.0 b

DMS: 4.482. Means with different letters in the column are statistically distinct (p<0.05).

same efficiency, so its use depends on the costs and the availability of plant material, since these plants can only be obtained in the wild during the summer. Aldás, 2014, mentions that applying neem oil from commercial formulations with 4.5 ml l^{-1} of water sprayed every 14 days produced 57.5% mortality of whitefly in open field chard crops. In this work, the field application was 5 ml of non-diluted formulated mixture per plant, achieving 60 and 62% efficiency in mortality in tomato and strawberry crops, respectively. López and Estrada, 2005, demonstrated that with the use of Oleo Nim 80 CE®, Neo Nim 60 CE®, Cuba Nim T®, Cuba Nim SM® and Foliar Nim HM® it is possible to effectively control damage caused by pests such as *Bemisia tabaci* (Genn.) in beans and tomato. The biological effectiveness achieved in these experiments ranged between 75 and 100%, which confirms the feasibility of the integration of these bioinsecticides in integrated pest management (IPM) for sustainable agriculture. In this work, it was necessary to emulsify the neem oil with nopal pectin and a lechuguilla-based adherent, which demonstrated greater adherence of the formulation on tomato leaves. Muñiz *et al.*, 2016, found that the products that showed the highest mortality were Neem Oil Spray and PHC® Neem, at a concentration of 0.6 mg ml^{-1}. The best repellent effect was from Neem Oil Spray (82.6%), PHC® Neeem (72.3%), Biosave® Neem (70.8%), and Neemix® 4.5 (59.9%); the first two showed greater persistence with similar effects after 3 days. In this study, mortality effects on whiteflies appeared after 72 hours of exposure to the formulation in a bioclimatic chamber and after 8 hours in greenhouse conditions. Santiago *et al*., 2009, evaluated the essential oils of cinnamon (*Cinnamomum zeylanicum* Breyne (Lauraceae)), orange (*Citrus sinensis* L.), clove (*Eugenia caryophyllata* Thumb) and thyme (*Thymus vulgaris* L), and conducted bioassays in greenhouses using the acrylic cylinder method, where 20 adults were exposed to a bean leaf disc treated with essential oil. Repellency was measured by the difference between insects perched and not perched on the disc at three, four, five, six and twenty-four hours after application. Cinnamon and thyme essential oils at 1% concentration showed the highest repellency (91 and 93%, respectively), clove essential oil was not very efficient, while orange essential oil did not cause repellency. Thyme oil was stable up to 24 hours. In this work, the spraying was done directly in a bioclimatic chamber as well as in a greenhouse, the application was at an undiluted concentration and applied directly. Santiago *et al*., 2013, used ethanolic extracts based on neem oil, chicalote, higuerilla and epazote of zorrillo to eliminate whitefly from tomato leaves and found that any of the extracts used exhibited a mortality of 76%, in this work however; none of the extracts surpassed 65% mortality in greenhouse conditions. Lopez and Estrada, 2005, used mixtures with water and different concentrations of neem oil against whitefly in cucumber crops in greenhouse conditions and reported mortality above 75% using a 20% concentration. Neem oil mixed with water did not emulsify properly in this work, it was necessary to apply an emulsifier. Cruz, 2009, mentions that ethanolic extracts showed insecticidal effects against whitefly eggs and nymphs, but not against adults. In eggs, ethanolic extracts of *Annona squamosa*, *Cryptandra myriantha*, *Petiveria*, *Alliacea* and *Tamarix arborea* when applied at 5 mg ml^{-1} caused more than 80% mortality.

In whitefly nymphs, ethanolic extracts of *Eugenia winzerlingii* and *P. alliacea* at 2.5 mg ml^{-1} caused more than 80% mortality. On the other hand, aqueous extracts only showed insecticidal effects against whitefly eggs, with extracts from *A. squamosa* and *E. winzerlingii* at a concentration of 0.75% causing more than 80% mortality. These results suggest that *A. squamosa* and *E. winzerlingii* species could be used in the future for natural control of *Bemisia tabaci*. No ethanolic extracts were used in this study; extraction was conducted with hot water in all cases, with the exception of neem oil, which was used as a commercial product.

CONCLUSIONS

Any form of extract prepared from neem oil, chicalote or skunkweed epazote, exhibits good results for the control of white mosquito in tomato and strawberry greenhouses, mixture preparation should be as described in this article. The bioformulation based on neem oil, chamomile extract and guishe extract, used periodically on vegetable crops in protected agriculture, represents a considerable reduction in the use of agrochemicals for the control of whiteflies, thrips and other leaf pests. The differences found in the percentage of mortality in greenhouses and in bioclimatic chambers are due to the fact that in the latter, photoperiod, relative humidity and temperature can be controlled more effectively.

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Colegio de Postgraduados

Gas production and environmental impact indicators from *in vitro* fermentation of diets with nopal silage (*Opuntia ficus-indica* L.)

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ABSTRACT
Objective: To evaluate the global warming potential index (GWPI) and in vitro gas production (GP) of fattening diets in lambs fed with silage of agricultural by-products of nopal cladodes and prickly pear (*Opuntia ficus-indica* L.) - hibiscus grain (*Hibiscus sabdariffa* L.) - oats straw (*Avena sativa* L.).
Design/methodology/approach: The GP technique was used to obtain the GWPI of isoproteic (crude protein (CP)) and isoenergetic diets (15% CP and 2.8 Mcal ME (metabolizable energy)) without silage (DWS; control), with corn silage (CSD) and with 10 or 20% of nopal-prickly pear-hibiscus grain-oat straw silage (DEN10, DEN20), fed for 60 days to 24 Creole fattening sheep.
Results: *In vitro* dry matter digestibility at 72 h ($DIVMS_{72}$) was better in CSD, but similar for DEN10, DEN20, and CSD. DEN10 and DEN20 had the lowest CH_4 production, GWPI, and environmental impact index (EII). The low fermentable fraction (LF; GP=24-72 h) was related to $DIVMS_{72}$.
Findings/conclusions: The cactus pear-hibiscus grain silage inclusion (DEN10, DEN20) in conventional diets had no effect on $DIVMS_{72}$, but decreased CH_4 emissions and the GWPI.

Keywords: Greenhouse effect gases, environmental impact, *Opuntia-Hibiscus*-straw by-products, *in vitro* gas production technique.

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INTRODUCTION

Ruminant production systems must have diverse ingredients that are a good source of nutrients, and as far as possible, reduce greenhouse gas emissions, [1], [2]. Given the scarcity and food prices increase, the utilization of agricultural by-products is an alternative to partially replace conventional sources of energy and protein used in diet formulations [3]–[5]. However, the energy and protein of these agricultural by-products do not always cover the requirements of the animals and create an imbalance in the rumen flora, generating higher carbon dioxide (CO_2) and methane (CH_4) production [1], [6], [7]. Incomplete fermentation of structural carbohydrates represents up to 10% of energy loss from foo, [8] having an environmental impact, since CH_4 is considerably more polluting than CO_2 [9].

The CH_4 and CO_2 emission estimation was carried out with *in vivo* techniques such as sulfur hexafluoride (SF6), and relatively expensive breathing chambers [10]. At the same time, ruminal fermentation CH_4 emission can be derived from an *in vitro* gas production technique, which is low-cost and less polluting [11], given that ruminant productive behaviour depends on the digestibility, fermentation, and nutritional contribution of their food, such as the energy: protein ratio [12], [13]. Recent studies have tested this technique to determine the potential production of greenhouse gases [14], [15].

The objective of this research was to evaluate the global warming potential index (GGWPI) and the in vitro gas production (PG) of fattening diets for sheep fed with nopal cladode-prickly pear-hibiscus grain-oatmeal straw silage.

MATERIALS AND METHODS

Location. The research was conducted at the sheep module of the Experimental Farm and the Livestock Microbiology Laboratory of the Universidad Autónoma Chapingo.

Silage. Except for the hibiscus grains, all ingredients were chopped (<2.5 cm). For the silage, a 64% nopal cladode, 11% prickly pear, 10% hibiscus grain, and 15% oat stubble mixture was compacted in 200-liter plastic drums to a 650 kg m^{-3} density. Subsequently, the drums were covered, and hermetically sealed. The silage was stored 60 days before use.

Treatments. Correspond to four isoproteic (15% CP) and isoenergetic (2.8 Mcal of ME kg^{-1}) diets: diet without silage (DWS, control), diet with corn silage (CSD), and diets with 10 or 20% of nopal cladode-prickly pear-hibiscus grain-oatmeal straw silage (DEN10 and DEN20) (Table 1). These diets were fed for 60 days to 24 creole male sheep (26.9±3 kg BW MS), housed in individual pens, and randomly assigned to one of the four diets (n=6). Between 30-45 and 46-60 d, three samples of the supplied sheep Diets were dried (DS), grounded, and used for *in vitro* fermentation.

Gas production kinetics and *in vitro* digestibility at incubation 72 h. The diet samples were fermented, and their produced gas was assessed via the GP technique [16], [17] following a modified and described procedure [15]. The maximum volume (Mv; mL g^{-1}), rate (S; h^{-1}), and the Lag phase (L; h) of the GP were estimated using a one-phase function [18] optimized with the SAS statistical software [19]; also, the dry matter degradability ($DIVMS_{72}$), calculated from the initial DS and the residual DS.

Also, the fast, medium, slow, and total fermentation fractions (FFF, MFF, SFF, TFF; g kg^{-1}) of the food were also obtained, transforming the accumulated gas volumes in the 0 to 8, 8 to 24 h, and 24 to 72 h of incubation intervals and the linear regression models described by [15].

Environmental impact indicators and *in vitro* digestibility after 24 hours of incubation. A modification to the GP methodology described was followed to determine the degradability ($DIVMS_{24}$), total gas volume (VOLT; mL g^{-1}), and methane

Table 1. Diets ingredients and nutritional composition.

Ingredient %	**Diet**			
	DSE	**DEM**	**DEN10**	**DEN20**
Oat straw	16	10	9.5	3
Ground corn	57.3	56.6	53	39
Rolled corn	-	-	-	10
Soybean paste	13.6	13.8	13	12.2
Corn Gluten	7.6	4.1	9	10.3
Mineral mix[†]	2	2	2	2
Calcium	2	2	2	2
Urea	0.5	0.5	0.5	0.5
Salt	1	1	1	1
Corn silage	-	10	-	-
Cactus-Prickly pear-ibiscus silage	-	-	10	20
Aporte nutrimental				
DM%[§]	89.7	74.2	74.7	73.3
ME (Mcal/kg^{-1})	2.8	2.8	2.8	2.8
CP%	15	15	15	15
NDF%	23.5	23.9	20.7	21.3

[†] Vitasal ovino plus: calcium, phosphorus, magnesium, sodium, chlorine, potassium, sulfur, y antioxidants (24, 3, 2, 8, 12, 0.5, 0.5, y 0.5%, respectively); lasolacide, chromium, manganese, iron, zinc, iodine, selenium, and cobalt (2000, 5, 4000, 2000, 5000, 100, 30, y 60 ppm respectively); A, D, and E vitamins (500,000, 150,000, y 1000 UI, respectively). [§] DM, dry mater; ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber.

production plus minor gases (CH_{4+GM}). The latter was adjusted to theoretical methane (% CH_4) with a 0.77 factor [14, 20]. The CO_2 and CH_4 volumes were used to calculate the Global Warming Potential Indicator (GWPI) with the equation:

$$GWPI\left(\text{mL CO}_2\ \text{eq g}^{-1}\text{DS}\right)=\text{CO}_2\left(\text{mL g}^{-1}\right)+\left[\text{CH}_4\left(\text{mL g}^{-1}\right)\times 2.3\right].\ [21]$$

GWPI and total gas volume (VOLT) were used to calculate the environmental impact index $\left(EII\left(\text{CO}_2\ \text{Eq}\right)=GWPI\,/\,VOLT\right)$.

Both fermentations for 24 and 72 h of incubation were repeated over time. Rumen inoculum from Dorpper male sheep with rumen cannula was used, adapted for 20 d to a diet without silage (DWS).

Statistical analysis. Analyzes of variance (ANOVA) were performed using diets as a fixed effect and repetitions (Rep) within the experiment run time (Time) as random effects (Model 1). The probability (P) of the fixed effects, the coefficients of determination (R^2), and variation (CV; %) were done with the Proc GLM [19], and the adjusted probabilities of Rep (Time) and standard errors (SE) se with Proc Mixed [19].

Model (1)

$$Y = \mu + Rep(Time)_{ij} + Diet_k + \varepsilon_{ijk}$$

Where: Y=Mv, S, L, $DIVMS_{72}$, FFF, MFF, SFF, TFF, CH_4, GWPI, EII, $DIVMS_{24}$, VOLT; μ=general average; $Rep(Time)_{ij}$ =effect of the i^{th}-repetition within the j^{th} execution time of the experiment; $Diet_k$=k^{th} diet effect; and ε_{ijk}=random error.

The LsMeans mean comparison test [19] was performed and the difference between means was analyzed using DMS, considering the SE, P=0.05 significance value, and the degrees of freedom (DF; 95) of the model error.

Simple linear correlations were obtained between pairs of variables (r) (Proc Corr; [19]). The correlation validity was obtained through the P values; as well as the multiple linear regression models by Forward) of Stepwise (Proc Reg; [19]), considering the variables inclusion with P<0.15. The models' validity was analyzed considering the regressor variables (βi) contribution to R^2 and its Mallow PC value.

RESULTS AND DISCUSSION

Table 2 shows that the Mv fluctuated between 303.45 and 321.38 mL g^{-1} at 72 h of incubation. These data are like those obtained by Martínez-Loperena *et al.* [22] for conventional diets, and by Vázquez-Mendoza [23] for corn or nopal silage diets; however, lower than those reported by Lazalde-Cruz [24] when 5 to 25% hibiscus seed (410.17 ml g^{-1}) was included. The $DIVMS_{72}$ varied from 79.16 to 81.77 %, higher than that reported by Miranda-Romero *et al.* [15] (52.3 to 54.8%) and by Muciño-Castillo [25] (61.44 to 69.55%), which suggests that the here evaluated diets show high degradation potential.

There were differences between Mv and $DIVMS_{72}$ diets (P<0.0001). DWS had higher Mv (321.38 ml g^{-1}) than DEN10 and DEN20 (P<0.05). The $DIVMS_{72}$ of the CSD was higher compared to DEN10 (P<0.05), but equal to DWS and DEN20 (P>0.05). DEN10 and DEN20 had the same degradation potential, but lower fermentative potential (Mv) than DWS.

The S was not different between diets, but DWS and CSD had higher L than DEN10 and DEN20 (P<0.05). Furthermore, the L of DEN20 was the lowest (P<0.05), indicating that increasing this silage proportion in the diet reduces the lag time. Higher L values were reported by Jiménez-Santiago *et al.* [27], Muciño-Castillo [26] values of 5.15 to 8.35 h when evaluating diets with nopal flour, while Lazalde-Cruz [24] reported L=3.91 h, in diets with hibiscus grain. Muciño-Castillo [26] and Lazalde-Cruz [24] reported 0.04 and 0.036 h^{-1} S values, like this research, which had no differences between diets.

Table 2 also shows FFF, MFF, SFF, and TFF values. The FFF was different between diets (P<0.002). Although previously studies reported similar values [15], [28], it increased as cactus silage in the diet increased (P<0.05), suggesting higher nonstructural carbohydrate content (NSC) previously related to the FFF [29].

The DWS, DEN10, and DEN20 had lower MFF than CSD ($P<0.05$). MFF is related to starch content [29]. CSD may have had a higher content of fermentable starch (a result of silage) than DEN10 and DEN20, which have fewer starch sources; including cactus silage tends to decrease MFF [15]. However, the MFF values of the present research were similar to previous ones when evaluating diets with cassava [28] and hibiscus [24]. The SFF was higher in DWS and CSD compared to DEN10 and DEN20 ($P<0.05$). The SFF is associated with cellulose content [29]. DEN10 and DEN20 could have lower NDF than DWS and CSD due to their oat straw and ME contents (28-37%; [23] *vs.* ≥59% [30], [31]).

DEN10 and DEN20 had lower TFF in DWS ($P<0.05$), which is again attributed to the 10% hibiscus grain inclusion in the EN, as a non-fermentable energy source in the rumen [32]. The TFF values are similar to those reported in diets with corn and nopal silage by Miranda-Romero *et al.* [15].

Table 3 shows the values for the environmental impact variables (CH_4, GWPI, EII), $DIVMS_{24}$, and VOLT were different among the diets ($P<0.0001$). The CH_4% values were similar to those of previous research in which whole grain high-concentrate diets were evaluated [14], [24], [33]. The CH_4%, GWPI, and EII were lower in the DWS than in the silage diets ($P<0.05$) and decreased when the proportion of EN increased. The IPGC and EII include the CH_4 volume (mL g^{-1} DS) in their calculation, which allows us to better understand the impact that diets can have on global warming (IPGC; mL CO_2 eq g^{-1} DS) in relation to the VOLT, which was similar in DWS, CSD, and DEN10 ($P>0.05$).

Studies mention that the CH_4% increase with the increasing low digestibility fiber in the diet [34], in this research DWS had better $DIVMS_{24}$, but due to its lower content of oat straw, diets with silage decreased the EII (*i.e.* DEN20 decreased 1.02 times the EII). When comparing Mv, $DIVMS_{24}$ and $DIVMS_{72}$, it was observed that during the first 24 hours, 86.5% of the potentially digestible dry matter is digested, and 64.5% of the total gas is produced (72 h).

Table 2. Gas production kinetics, *in vitro* dry matter (DM) degradability, and fermentable fractions of sheep diets with corn silage and nopal silage.

Tratamiento	Mv mL g^{-1}	S h^{-1}	L h	$IVDMD_{72}$ %	FFF	MFF	SFF	TFF
					ml g^{-1} DM			
DSE†	321.38 ^{a}	0.0408 ^{a}	3.57 ^{a}	80.10 ^{ab}	179.85 ^{b}	263.63 ^{ab}	289.63 ^{a}	733.12 ^{a}
DEM	316.20^{ab}	0.0412 ^{a}	3.85 ^{a}	81.77 ^{a}	172.94 ^{b}	272.84 ^{a}	280.69 ^{a}	726.47 ^{ab}
DEN (10%)	303.45 ^{b}	0.0400 ^{a}	3.24 ^{b}	79.16 ^{b}	180.64 ^{b}	253.75 ^{b}	260.27 ^{b}	694.67 ^{bc}
DEN (20%)	303.77 ^{b}	0.0404 ^{a}	2.71 ^{c}	80.36 ^{ab}	200.13 ^{a}	249.57 ^{b}	242.59 ^{b}	692.30 ^{c}
Valor de P	<0.0001	<0.13	<0.0001	<0.0001	<0.002	<0.0001	<0.0001	<0.0001
R^2	0.55	0.35	0.53	0.66	0.48	0.64	0.70	0.60
VC (%)	9.11	7.43	19.38	5.18	13.24	7.69	14.45	0.34
SE	8.19	0.0009	0.19	1.20	7.01	5.77	11.19	19.19
DMS 0.05=	13.68	0.0015	0.31	2.01	11.71	9.64	18.70	32.07

† Vm, maximum volume; S, rate of gas production; L, time Lag; $IVDMD_{24}$, *in vitro* dry mater digestibility 24 h; FFF, MFF, SFF, y TFF, fast, medium, slow and total fermentable fraction, respectively; DSE, diet without silage; DEM, diet with corn silage; DEN, diet with cactus silage; R^2 y VC determination and variation coefficient ; SE, standard error; [a,b,c] averages in the same column with different literals are different.

Table 3. *In vitro* atmospheric impact variables and dry matter digestibility (DM).

Tratamiento	CH_4 %	GWPI $mLCO_2$ eq g^{-1} MS	EII CO_2 eq	$DIVMS_{24}$ %	VOLT mL g^{-1} MS
DSE[†]	22.16 [a]	1601.64 [a]	5.81 [a]	71.30 [a]	205.33 [a]
DEM	20.54 [b]	1450.41 [b]	5.45 [ab]	69.70 [ab]	203.69 [a]
DEN (10%)	19.12 [c]	1243.18 [c]	5.15 [bc]	68.53 [b]	203.06 [a]
DEN (20%)	17.48 [d]	1187.92 [c]	4.79 [c]	68.46 [b]	190.52 [b]
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R2	0.6297	0.5782	0.6299	0.554	0.6222
VC (%)	20.12	21.52	16.32	5.36	6.58
EE	1.15	85.16	0.24	1.07	3.81
DMS 0.05=	1.9216	142.3023	0.4010	1.7879	6.3665

[†] CH_4, % theorical methane; GWPI, global warming potential index; EII, Environmental impact indicator: VOLT, total volume of CH_4; DSE, diet without silage; DEM, diet with corn silage; DEN, diet with cactus silage; R^2, determination coefficient; VC, variation coefficient; SE, standard error; [a,b,c] averages in the same column with different literals are different.

Pearson correlations. Table 4 shows the simple correlations between the variables evaluated in this research. The highest FFF value relates to a decrease in L (r=−0.75; P<0.0001). Higher FFF and Mv relate to better $DIVMS_{24}$ (r=0.26 to 0.37; P<0.01) and VOLT with $DIVMS_{72}$ (r=0.48; P<0.0001). Also, degradability may have similar trends after 24 and 72 h of incubation (r=0.46 $DIVMS_{24}$ with $DIVMS_{72}$; P<0.0001). VOLT negatively correlated with the CH_4 % (r=−0.28; P<0.01), since the highest VOLT comes from the soluble carbohydrates and starch fermentation (0-24 h), expecting less CH_4 production [34].

Table 4. Correlations between the potential global warming index variable calculation via the *in vitro* gas production technique.

	GWPI	VOLT	CH_4	IVD_{24}	MV	S	L	FFF	MFF	SFF	TFF	IVD_{72}
EII	0.83^{***}	-0.28^{**}	0.99^{***}	-0.13_{NS}	-0.12_{NS}	-0.13_{NS}	0.07_{NS}	-0.28^{**}	-0.29^{**}	0.08_{NS}	-0.13_{NS}	-0.14_{NS}
GWPI		-0.01_{NS}	0.83^{***}	-0.26^{**}	-0.08_{NS}	-0.07_{NS}	0.21^{*}	-0.37^{**}	-0.22^{*}	0.16_{NS}	-0.08_{NS}	-0.11_{NS}
VOLT			-0.28^{**}	0.18_{NS}	-0.05_{NS}	0.31^{**}	0	0.08_{NS}	0.06_{NS}	-0.19_{NS}	-0.08_{NS}	0.48^{***}
CH_4				-0.13_{NS}	-0.12_{NS}	-0.13_{NS}	0.07_{NS}	-0.28^{**}	-0.29^{**}	0.08_{NS}	-0.13_{NS}	-0.14_{NS}
$IVDI_{24}$					-0.24^{**}	0.182_{NS}	-0.35^{**}	0.26^{**}	-0.15_{NS}	-0.47^{***}	-0.29^{**}	0.46^{***}
MV						-0.43^{***}	0.22^{**}	0.37^{**}	0.84^{***}	0.85^{***}	0.96^{***}	-0.25_{NS}
S							0.06_{NS}	-0.19_{NS}	-0.23^{*}	-0.54^{***}	-0.50^{***}	0.36^{**}
L								-0.75^{***}	0.41^{***}	0.43^{***}	0.18_{NS}	-0.10_{NS}
FFF									0.26^{**}	0.01_{NS}	0.41^{***}	0.10_{NS}
MFF										0.67^{***}	0.85^{***}	-0.05_{NS}
SFF											0.89^{*}	-0.51_{NS}
TFF												-0.32^{**}

EIIA, Enviromental impacto indicator; GWPI, global warming potential index; VOLT, total volume; CH_4, % theorical methane; $IVD_{24, 72}$, *in vitro* dry mater degradation 24 and 72 hours; MV, MAXIMUN volume; s, rate of gas production; L, lag time; FFF, fast fermetable faction; MFF, medium fermentable fraction; SFF, slow fermentable fraction; TFF, total fermentable fraction. ***P<0.0001; **P<0.01; *P<0.05; NS, P>0.05.

Better FFF and MFF could also relate to greater degradability at 24 and 72 h and lower GWPI ($r=-0.22$ to -0.37 GWPI with FFF, MFF, and $DIVMS_{24}$; $r=-0.25$ to $r=-0.26$ CH_4 with FFF and MFF; $P<0.05$) which corroborates that reported by Rasmussen and Harrison [34]. Although SFF positively correlated with Mv ($r=0.85$; $P<0.0001$), it negatively correlated with $DIVMS_{24}$ ($r=-0.47$; $P<0.0001$), likewise, $DIVMS_{72}$ also negatively correlated with TFF ($r=-0.32$; $P<0.01$).

Multiple linear regression models. Consistently, the SFF variable would allow obtaining the $DIVMS_{72}$ (Y) ($P<0.0001$). When including all diets: $Y=93.92-0.05\ SFF+\varepsilon_{ij}$ ($R^2=0.26$); DWS: $Y=94.7-0.05\ SFF+\varepsilon_{ij}$ ($R^2=0.34$); and CSD $Y=105.53-0.085\ SFF+\varepsilon_{ij}$ ($R^2=0.77$). In DEN10 and DEN20 the VOLT contributed 0.16 and 0.20 to the R^2 ($P<0.0001$) [$Y=11.6+0.63\ DIVMS_{24}+0.13\ VOLT+\varepsilon_{ij}$ ($R^2=0.42$); $Y=47.67+0.16\ VOLT+\varepsilon_{ij}$ ($R^2=0.20$)]. The amount of fiber contained in diets relates to gas production [15].

CONCLUSIONS

The diets containing the silage composed of nopal cladode-prickly pear-hibiscus grain-oatmeal straw showed the same degradability potential as the control diet and were similar to the diet with corn silage. The accumulated volume of gas at 24 h negatively correlated with the methane percentage, therefore, diets high in concentrate and low in fiber have lower methane emissions. The diet with silage mainly composed of nopal cladode and prickly pear provides a greater amount of non-structural carbohydrates and more degradable fiber, which reduced the average fermentable fraction, as well as the environmental impact index 1.01 times. The slow fermentation fraction could predict the degradability at 72 h. According to these results, including this compound silage would not negatively affect the productive behavior of fattening lambs.

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Colegio de Postgraduados

Phytopathogenic fungi associated with blueberry dieback (*Vaccinium corymbosum* L.) pruning and sealing management

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ABSTRACT

Objective: To identify the phytopathogenic fungi related to blueberry dieback, verify their pathogenicity, incidence after pruning, and the type of sealing.

Design/methodology/approach: For this, symptomatic stem and branch samples were collected in eight commercial blueberry lots at the Ahome, El Fuerte, and Guasave municipalities, state of Sinaloa, from which 196 fungal isolates were obtained. These were morphologically identified to subsequently perform detached leaf and twig pathogenicity tests, on the Biloxi variety; likewise, two pruning angles and three sealants were evaluated and compared to an absolute control in a completely random arrangement.

Results: Based on morphological analysis the *Alternaria*, *Fusarium*, *Lasiodiplodia*, *Pestalotia*, and *Curvularia* genera were detected. However, *Lasiodiplodia* isolates were pathogenic in leaves and twigs, while the best result is achieved with the angle 45° pruning sealing with washable plastic-type white vinyl paint plus copper oxychloride.

Limitations on study/implications: None.

Findings/conclusions: The results open new research lines related to molecular identification and disease impact on performance.

Keywords: blueberry, dieback, *Lasiodiplodia*.

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INTRODUCTION

Blueberry (*Vaccinium corymbosum* L.) is a fruit plant, recently grown in at least 30 countries. Peru (261,450 t), Chile (185,300 t), Mexico (85,100 t), United States (328,210 t), South Africa (26,000 t), Poland (55,500 t) and Canada (80,420 t) stand out as suppliers (USDA-FAS, 2021). In Mexico, job creation during its harvesting season is highlighted, since it is a 100% manual labor process with a significant economic impact (Pérez-Cruz, 2018). From planting to harvest, blueberries require technical support, constant care, and different conditions, coupled with intensive agricultural practices. This has induced emerging phytopathogens that impact production. Worldwide, various fungi have been

reported to induce damage in different producing areas, among which *Alternaria* sp., *Curvularia* sp., *Microsphaera vaccinii*, *Phomopsis vaccinii*, *Stemphyllium* sp. (Cline and Schilder, 2006), *Bipolaris cynodontis* (Sisterna *et al*., 2009), *Botrytis cinerea* (Bristow and Milholland, 1995) stand out. One of the most common diseases is the so-called "descending death blueberry dieback", caused by members of the Botryosphaericeae family, particularly *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, and *Neofusicoccum parvum*. These, without exception, colonize all stem tissues, inducing leaf and stem necrosis due to a lack of water and nutrients. The above makes knowing the pathogens present in commercial plantations necessary to establish management strategies, and these phytopathogens' correct identification and characterization essential. Therefore, this research objectives were a) to identify the fungi related to blueberry dieback through morphological studies; b) *in vitro*, determine the pathogenicity of fungi associated with blueberry dieback; c) to evaluate *in planta* two pruning and sealing techniques used in the region, and their correlation to blueberry dieback incidence.

MATERIALS AND METHODS

Seven commercial orchards of between two and four hectares were sampled at the Guasave, El Fuerte, and Ahome municipalities (state of Sinaloa) from October 2020 to July 2021; sampling in zigzag, 28 symptomatic stems were taken per orchard. The collected samples were transported to a laboratory in humid chambers at 4 °C. Isolation was performed in water-agar (AA; Bioxon; Cuautitlán Izcalli, State of Mexico, Mexico) following the procedure by Maraite *et al*. (1997) with a modification. To purify the isolates, hyphal tips were transferred to potato-dextrose-agar (PDA; Bioxon; Cuautitlán Izcalli, State of Mexico, Mexico). The pure culture media were preserved in filter paper, 10% glycerol, and three-times-distilled sterile water (TDSW), for later use.

The morphological characterization was done by taking macroscopic variables such as the colony color, mycelium type, acervuli, pycnidia, sporodochia, ascostromas, free conidiophores, and conidia presence; in addition to their radial growth rate (Granados-Montero, 2018). The pure isolates were placed in Petri dishes with PDA medium and incubated for 12 days at 25±2 °C in a 12 h light by 12 h dark regime, to determine their phenotypic characteristics and assess the mycelial growth rate (French and Hebert, 1980). The microscopic characterization was done with an Olympus Lx compound microscope with a micrometer. The shape of the conidia, the number of cells, coloration, length, width, and ornamentations were also considered. The production of pigments, type of margin, texture, and density were determined by reports in the literature, and the colony color, on the front and back, based on the color scale by Kelly and Judd (1976). The detached leaf pathogenicity occurred in the Biloxi varieties, on the first run, and in the Atlanthis during the second. Well-developed leaves were taken from the middle part of the plants. Inoculum production was done following the methodology by Foolad *et al*. (2000) with some modifications. Pathogenicity tests on detached leaves were carried out following the methodology by Peever *et al*. (2000) with some modifications. The experiment was carried out on two occasions, their treatments were distributed in a completely random arrangement with four repetitions and an absolute control.

Pathogenicity was evaluated seven days later by observing and measuring the affected leaf area (ALA). The pathogenicity tests on twigs were assessed in healthy 12 cm twigs with no leaves and disinfected with a 1.25% sodium hypochlorite solution. For this, 5 mm diameter mycelium discs from the colony's growth margin were placed in the center of pre-punctured twigs, then, incubating four twigs per tray in a humid chamber. The experiment was run twice, under normal light and temperature conditions for nine days, with a random distribution; Affected length area was the evaluated parameter (De la Mora-Castañeda *et al.*, 2014). The *in planta* tests were done on two-year-old Atlanthis variety plants, evaluating two pruning angles, 180° and 45° in a three sealants combination (Berel brand, washable plastic-type white vinyl paint, washable plastic-type white vinyl paint brand Berel plus copper oxychloride at a rate of 100 g per L of paint, and hydrogen peroxide), compared to plants with no sealing, in four repetitions; The experiment ran for 60 days in a random arrangement, under natural conditions of light, humidity, and temperature.

The affected leaf area percentages were statistically analyzed using the Kormogorof-Smirnov normality test and applying the Lilliefors correction, before an ANOVA using the SPSS Statistics 26 software. Given that the experiments were run on two occasions, and these showed an interaction between isolates and experiments, both results are expressed together.

RESULTS AND DISCUSSION

The colony and conidia phenotypic characteristics were determined in PDA after the corresponding incubation period. This allowed the identification of isolates (Figure 1) in the *Alternaria* spp, *Fusarium* spp, *Lasiodiplodia* spp, *Pestalotia* spp., and *Curvularia* spp. genus (Barnett and Hunter, 1972 and Phillips *et al.*, 2013).

From the total obtained isolates, characteristics from several genera were observed in different percentages (Figure 2), *Alternaria* spp. the most frequent.

In the detached leaves, not all the collected isolates were pathogenic. There are significant differences between isolates, $\alpha=0.05$, F=2.344, and 95% CI. The most pathogenic

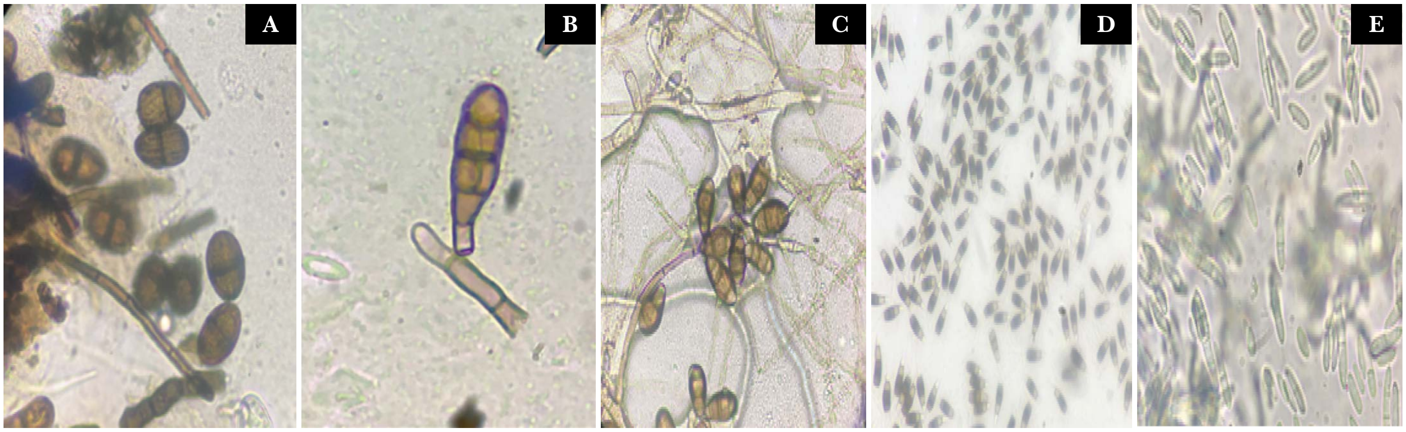

Figure 1. Conidia from PDA culture medium. A) *Lasiodiplodia* spp; B) *Alternaria* spp; C) *Curvulary*; D) *Pestalotia* spp, and E) *Fusarium* spp.

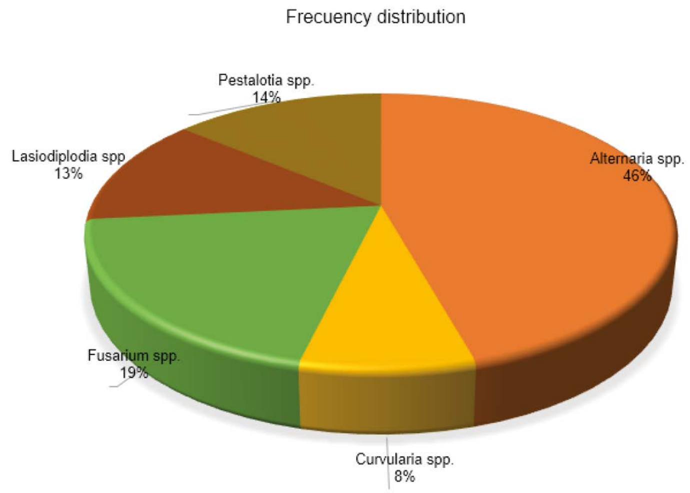

Figure 2. Pathogens percentage by gender.

being *Lasiodiplodia* spp. and *Pestalotia* spp. (Figure 3a) in their tests. Meanwhile, in the pathogenicity tests on vareta, only the isolates corresponding to the genus *Lasiodiplodia* spp. were pathogenic (Figure 3b).

The first in planta symptoms in the tests occurred between days 45 to 60 and appeared from the cutting area. There are significant differences between the tested treatments, vinyl paint plus copper oxychloride treatment the one with the best results.

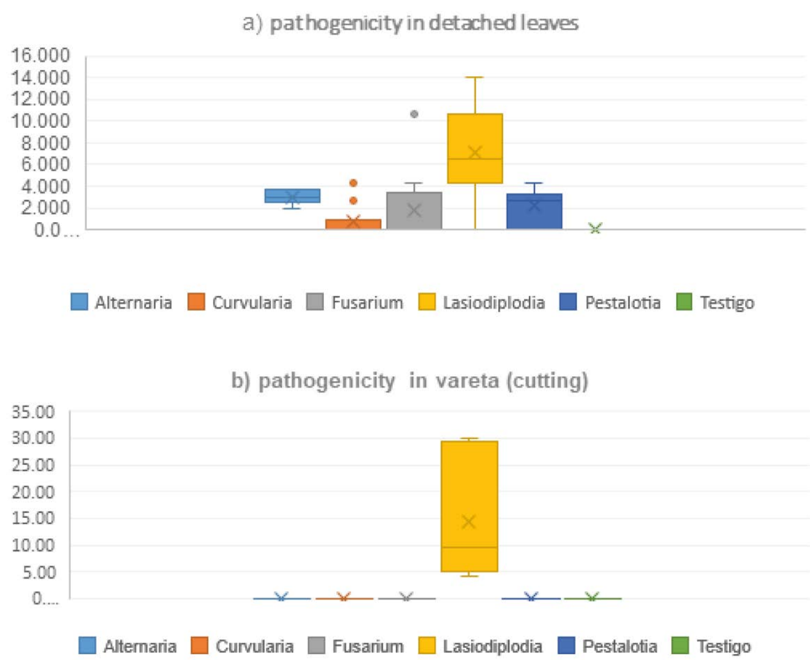

Figure 3. Pathogenicity tests. a) pathogenic isolates in detached leaves; b) pathogenicity in twigs (*Lasiodiplodia* spp.).

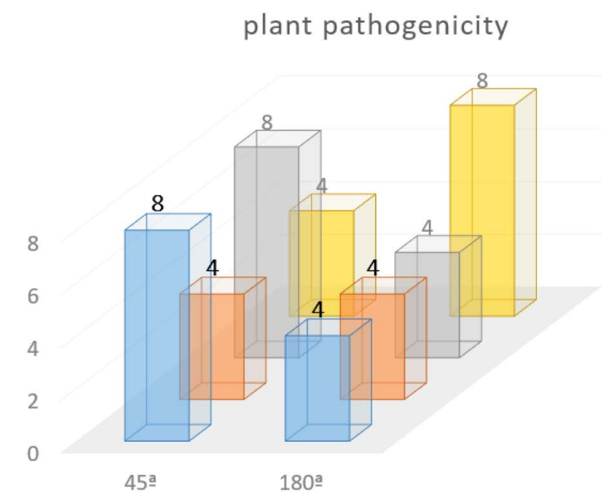

Figure 4. Plants with initial symptoms of blueberry dieback 60 days after pruning: blue bar: vinyl Paint; red bar: vinyl Paint + copper; yellow bar: unsealed

CONCLUSIONS

The pathogens of *Alternaria* spp., *Fusarium* spp., *Lasiodiplodia* spp., *Pestalotia* spp. *Curvularia* spp. and *Botryosphaeria* spp. genera were found associated with blueberry dieback, which occurs due to the different pruning on the crop. The identity of the species was done through morphological characterization. From the total tested isolates, only those from the *Lasiodiplodia* spp. genus correlate with the leaf and rod pathogenicity tests. Likewise, the best treatment to seal, with any pruning angle, is vinyl paint plus copper oxychloride at a 100g/L rate.

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Colegio de Postgraduados

Essential Oil of Eucalyptus: a natural solution for treating pediculosis

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ABSTRACT

Objective: Pediculosis is a condition caused by the infestation of *Pediculus Humanus Capitis*. The pesticides used in current formulations exhibit toxicity and carcinogenic effects on consumers. This study aimed to investigate the pediculicidal activity of the essential oil from *Eucalyptus globulus* leaves, with the intention of adding it as an active ingredient in pediculicidal formulations to replace harmful chemicals.

Design/methodology/approach: *In vivo* tests were conducted to assess the repellency and mortality of the essential oil obtained through hydrodistillation. The major components were determined through gas chromatography-mass spectrometry analysis. Additionally, proximate, and chemical composition analyses were performed on the eucalyptus leaves using ASTM E871, ASTM E872, ASTM D1104, TAPPI T264, TAPPI T207, ASTM D1106, and ASTM D1104 methods.

Results: A repellency of 66.66% and 100% mortality within 2.26 minutes were obtained in the *in vivo* tests. The yield of hydrodistilled essential oil was 4 mL/kg, primarily composed of 71.04% 1,8-cineole, 18.94% 4-Ethyl-m-xylene, 2.72% γ-Terpinene, and 1.23% L-α-Pinene. Furthermore, the composition of eucalyptus leaves was determined as 61.25% moisture, 30.32% volatile matter, 6% ash, 2.40% fixed carbon, 11.22% acetone extractives, 33.03% water extractives, 31.49% lignin, 69.33% holocellulose, 62.09% cellulose, and 7.24% hemicellulose.

Limitations on study/implications: The pediculicidal activity study was conducted solely on the essential oil, and further testing on the formulation of the finished product is necessary.

Findings/conclusions: The pediculicidal activity study was conducted solely on the essential oil, and further testing on the formulation of the finished product is necessary.

Keywords: Essential oil, *Eucalyptus globulus*, pediculosis, physicochemical characterization.

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INTRODUCTION

Infestation of *Pediculus humanus capitis*, or head lice, is a chronic condition worldwide. In Mexicali, Baja California, Mexico, lice infestation is abundant due to the climatic conditions. Lice are six-legged arthropods that invade the host's hair in search of blood for feeding. The life cycle of head lice consists of three stages: eggs, colloquially known as nits, measuring 1 mm, are white and attached to the hair by a non-polar substance called cement; the second stage is nymphs, classified as such at birth, measuring less than 1 mm and being whitish; finally, the adult stage, where lice mature 2 to 3 weeks after birth, measuring between 3 and 4 mm, are brown in color, and live for 3 to 4 weeks, constantly feeding (Herranz & Abad, 2008). Primary sources of contagion are primary education schools. The trend indicates an increase in lice infestations in summer due to children's exposure to high temperatures, creating conditions for lice to reproduce rapidly. Various studies have shown that lice have developed resistance and immunity to conventional treatments for their extermination, such as pyrethrins, pyrethroids, malathion, carbaryl, and benzyl benzoate, in addition to confirming their toxicity in humans and potential carcinogenic effects (Burkhart & Burkhart, 2000; McCage, Ward, & Paling, 2002; Herranz *et al.*, 2008; Veracx & Raoult, 2012). Currently, organic alternatives are being studied to treat pediculosis without affecting patients' health. Experimentation with the effectiveness of essential oils as pediculicides and ovicides has been chosen, yielding favorable results in the total or partial elimination of head lice. Essential oils have the ability to deplete Adenosine Triphosphate (ATP) levels in lice, leading to asphyxiation and/or dehydration (McCage *et al.*, 2002; Pearlman, 2004).

Eucalyptus globulus essential oil is one of the most commonly used as an insecticide, supported by studies generated through gas chromatography, allowing for the qualification and quantification of the chemical composition of essential oils. *In vivo* tests on lice endorse the attributions of the pesticide effects to 1,8-Cineole, which causes inhibition of acetylcholinesterase, contact toxicity, reproductive function impairment, and fumigant action against *Pediculus humanus capitis* and other species. α-Pinene and β-Pinene also possess fumigant activity (Choi *et al.*, 2006).

MATERIALS AND METHODS

Physicochemical Characterization

Table 1 presents the procedures used for proximate and chemical composition analysis. Each method was performed in triplicate.

Pilot-scale Hydrodistillation

The hydrodistillation extraction at a pilot scale began by loading 1 kg of previously cut eucalyptus leaves. It was brought to a boil in the presence of 7.19 L of water, creating a steam-vapor mixture (water and essential oil) (Peredo, Palou & López, 2009). The water vapor and essential oil mixture passed through a condenser, where it was cooled to low temperatures using cold water to reach room temperature. The mixture transformed into an unstable liquid emulsion composed of essential oil and hydrosol. Finally, the emulsion underwent decantation, and it was easily separated due to immiscibility caused by the difference in density. The result was the essential oil as the main product and aromatic water as a byproduct. The equipment used is shown in Figure 1.

Repellency and Mortality Tests

Figure 2 displays the methods used in the effectiveness tests of the essential oil from eucalyptus leaves. Repellency and mortality were studied in nymphs, young lice, and adult lice. Both tests were conducted in triplicate.

Empty Petri dishes were used as controls, and Petri dishes with purified water were used as placebos, following the methodology outlined for each test.

Table 1. Physicochemical characterization.

No.	Analysis	Standard method	Procedure
1	Moisture	ASTM E871, (1998)	Drying in oven at 65 °C for 48 h
2	Volatile Matter	ASTM E872, (2013)	Drying in oven at 950 °C for 7 min
3	Ashes	ASTM D1102, (2001)	Drying in oven at 580 °C for 4 h
4	Fixed Carbon	-	Percentage difference
5	Solvent Extractables	TAPPI T264, (2007)	Acetone extraction using a Soxhlet apparatus for 8 h, followed by drying in an oven at 105 °C for 4 h
6	Water Extractables	TAPPI T207, (1999)	Fractional distillation with water at 100 °C for 4 h, followed by drying in an oven at 105 °C for 4 h
7	Lignin	ASTM D1106, (2001)	Agitation at 200 rpm for 2 h with 72% sulfuric acid, followed by fractional distillation with 3% sulfuric acid for 4 h. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
8	Holocellulose	ASTM D1104, (1985)	Addition of acetic acid and sodium chlorite every 5 h with agitation, maintaining a temperature of 75 °C. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
9	Cellulose	Rowell, (2012)	Addition of 17.5% sodium hydroxide every 5 minutes for 20 min. Addition of water and allowing to rest for 1 h. Dilution with 8.3% sodium hydroxide and 10% acetic acid. Filtration using a Büchner funnel with a No. 5 filter and vacuum pump. Finally, drying in an oven at 105 °C for 4 h
10	Hemicellulose	-	Percentage difference

Figure 1. Pilot-scale hydrodistillation apparatus by Armenta *et al*., 2023.

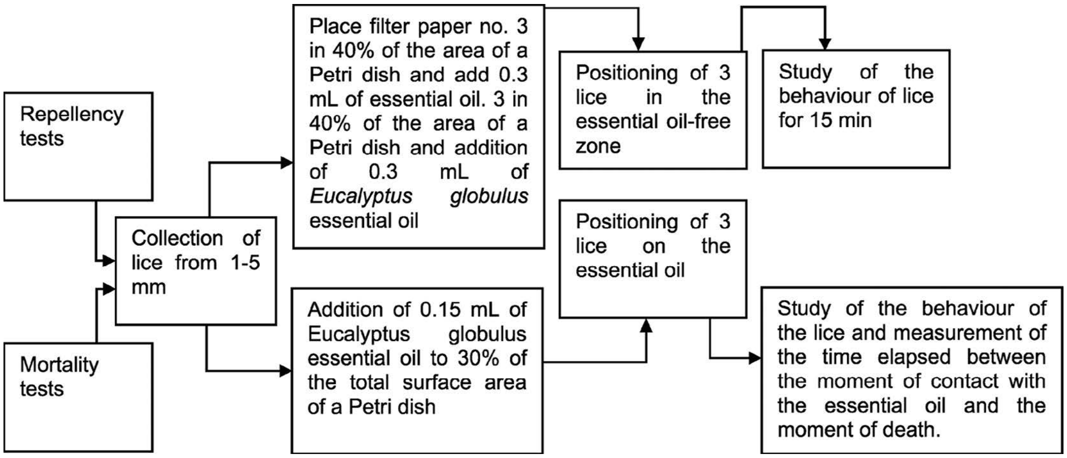

Figure 2. Methodology for determining pediculicidal activity.

Gas Chromatography-Mass Spectrometry

To determine the major components of the essential oil from eucalyptus leaves, gas chromatography-mass spectrometry (GC-MS) was used. The essential oil was characterized using an Agilent Technologies 7890A Network GC-MS system. Mass spectra were obtained by electron impact ionization at 70 eV energy. The initial temperature was set at 50 °C for 2 minutes and then increased at a rate of 5 °C/min until reaching 250 °C. Helium was used as the carrier gas, with an inlet pressure of 12.667 psi. Samples were prepared by dissolving 20 microliters of the essential oil in 980 microliters of dichloromethane. From the resulting solution, 1 microliter was taken for injection (Jaramillo *et al.*, 2022).

RESULTS AND DISCUSSION

Physicochemical Characterization

Table 2 presents the results of the proximate analysis and chemical composition analysis performed on *Eucalyptus globulus* leaves in Mexicali, Baja California.

The results were compared with another species of *Eucalyptus globulus* located in Morelia, Michoacán. Additionally, comparisons were made with *Eucalyptus pellita* and *Eucalyptus citriodora* species located in Cuba, as well as *Gossypium hirsutum* L and *Triticum aestivum* L, two endemic species of Mexicali. The moisture content is considered high, the ash content

Figure 3 shows the design of each test.

Table 2. Physicochemical Characterization of *Eucalyptus globulus* leaves.

Analysis	Content (%)	Standard deviation
Moisture	61.2598	0.3039
Volatile Matte	30.3263	0.2242
Ashes	6.0040	0.2271
Fixed Carbon	2.4097	0.0881
Acetone Extractables	11.2288	0.6186
Water Extractables	33.0398	0.6659
Total Extractable	44.2687	0.9716
Lignin	30.6632	0.3992
Holocellulose	69.3367	0.7414
Cellulose	62.0946	0.6336
Hemicellulose	7.2420	0.1340

is within the average range, while the volatile and fixed carbon contents are low. The results of acetone extractables are low, whereas the water extractables are considerably higher. The lignin content is moderately high, the holocellulose and cellulose contents are high, and the hemicellulose content is low.

Essential oil extraction

Table 3 presents the results of the essential oil extraction in a pilot-scale system.

GC-MS

The essential oil of *Eucalyptus globulus* leaves is mainly composed of 71.04% of 1,8-cineol, 18.94% of 4-Ethyl-m-xylene, 2.72% of γ-Terpinene, and 1.23% of L-α-Pinene (Armenta *et al.*, 2023).

Pediculicidal Activity

The results of the repellency tests showed an effectiveness of 66.66%, while the mortality tests exhibited a 100% effectiveness within an average time of 2.66 minutes. The essential oil of eucalyptus demonstrated outstanding mortality rates, eliminating lice in a shorter time compared to essential oils of *Citrus sinensis* and *Cymbopogon nardus*. In terms of repellency, all three essential oils showed similar results. During the tests conducted with the control group, the lice moved along the entire surface of the Petri dish for the duration of 15 minutes. In the placebo tests, for repellency, the lice explored the entire Petri dish, including the area with water, but found it difficult to move in that section. Once out of

Table 3. Results of eucalyptus essential oil extraction.

Plant material	m_{plant} (kg)	V_{water} (L)	V_{AE} (mL)	Yield ($V_{AE}/m_{vegetal}$)
Eucalyptus globulus	1	7.50	4.40	4.40
	1	6.58	4.60	4.60
	1	7.50	4.00	4.00
Mean	1	7.19	4.33	4.33

the water, they resumed normal activity. In the mortality tests, when the lice were placed directly on the water, they remained there for an average of 12 minutes. 83% of the lice managed to leave the water without apparent harm, while 17% remained in the water, showing signs of life throughout the entire test.

CONCLUSIONS

The physicochemical analyses conducted on *Eucalyptus globulus* indicate that it is a viable option for obtaining essential oils using physical conversion technologies such as steam distillation or hydrodistillation. The repellency and mortality tests confirmed the pediculicidal activity of the essential oil from *Eucalyptus globulus* leaves obtained through hydrodistillation. The essential oil of *Eucalyptus globulus* demonstrated favorable results in terms of its repellent and inhibitory properties within short time frames. These characteristics are mainly attributed to the high content of 1,8-cineol, followed by L-α-Pinene. It is recommended to develop a pediculicidal formulation based on this essential oil and conduct further tests to determine its lethal concentration once the formulation is completed.

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Colegio de Postgraduados

Comparing outstanding papaya lines for selecting and preserving improved characters

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ABSTRACT

Objective: To evaluate outstanding and adapted papaya lines derived from selection to conserve desirable characteristics.

Design/methodology/approach: 23 lines of the 'Maradol' type were evaluated at Antunez Michoacan, Mexico. Initially, the plants' height, stem circumference, number of leaves, and first fruit height were recorded. During their development, outstanding plants were identified, and their self-pollinating was promoted. In the fruits, their polar and equatorial circumference, shape index, weight, width and pulp firmness, and soluble solids were assessed.

Results: The plants' development was different, their variability between lines allowed identifying morphological characteristics of interest. Only 10 lines had this condition. The number of fruits formed over covered flower buds and collected fruits on formed fruits was reduced. The fruits' characterization, except for their soluble solids, showed differences. Multivariate analysis indicated variability associated with each principal component.

Limitations on study/implications: Currently in Mexico, there are few papaya varieties, the 'Maradol' variety being dominant, and vulnerable to phytosanitary problems over time. However, developing varieties and seed production is challenging and the pollination control of the plants necessarily intervenes.

Findings/conclusions: Out of 23 assessed papaya lines, only 43.48% reported outstanding plants. Inside these lines, between 5 and 10% of the plants were chosen. In the developmental progress from the covered flower buds' stage to formed and collected fruits, only 28% of fruits were obtained. The selected lines showed fruit variability.

Keywords: *Carica papaya*, 'Maradol' genotype, hermaphrodite, plant sexing.

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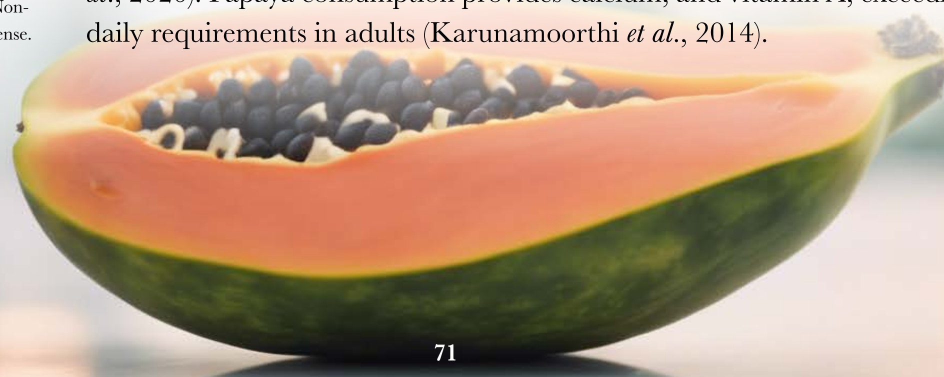
INTRODUCTION

Of the 22 species in the *Carica* genus, the papaya (*Carica papaya* L.), native to the American tropics, is the one with the greatest economic importance. It has traditionally been cultivated in different regions of America, Africa, Asia, Australia, countries like the Philippines, and the United States (Hawaii and Florida); and recently in Europe (Honoré *et al.*, 2020). Papaya consumption provides calcium, and vitamin A, exceeding the minimum daily requirements in adults (Karunamoorthi *et al.*, 2014).



Mexico ranked fifth among the countries with the largest established area, and fourth by production volume (FAOSTAT, 2021). During 2022, the harvested area in Mexico was 19,698 ha, mainly in Veracruz, Colima, Michoacán, Oaxaca, Chiapas, and Guerrero states. Particularly, Michoacán registered 3,135 ha, and 112,586 tons of production (SIAP, 2023). The papaya production activity generates direct and indirect jobs and boosts regional economies. In Mexico, the 'Maradol' variety is dominant (SIAP, 2017). For its propagation, the seed type varies from original "F1" to "Fn" selections. Given this, the possibility of degeneration exists, leading to a low number of offered varieties, adapted to the different agroecological regions of the country, and a marked imbalance in the materials resistance capacity introduced in the papaya-producing areas, making them vulnerable to pests and diseases. Also, this species has a complex floral biology, since it has female, male, and hermaphrodite plants (Damasceno *et al.*, 2018), which influences the fruit's production and quality. Thus, developing papaya varieties with enhanced agronomic traits, fruit quality, and high disease resistance levels is a challenge (Vivas *et al.*, 2017). Generally, papaya is an open-pollinated species (Urasaki *et al.*, 2012), which limits the uniform development of plantations in later periods.

In Mexico, the utilized varieties were originated by selection and improvement, where controlled pollination is key. Consequently, outstanding plants with some characteristics of interest are chosen, and their pollination is subsequently controlled. If crosses are made between plants, these should preferably be between hermaphrodite plants, or self-pollination should be promoted so that, depending on the floral proportion, 66% of their seeds are expected to originate hermaphrodite plants (Ram, 2005). Thus, using improved genotypes must meet criteria that influence the species productive potential and the appropriate environment for its development (Nunes *et al.*, 2018). Therefore, it is necessary to rescue genetic material that can be used in the improvement of papaya seed production and new materials development (Álvarez and Tapia, 2019), adapted to regions of interest (SNITT-SAGARPA, 2016). For this, genetic diversity research is important (da Silva *et al.*, 2017). Based on the above, different lines of outstanding papaya plants derived from selection in commercial environments were evaluated to preserve improved productive characteristics.

MATERIALS AND METHODS

Starting in 2022 at the town of Antúnez, state of Michoacán, Mexico, 23 outstanding papaya lines of the 'Maradol' type were experimentally evaluated. From these, 14 lines of selected plants came from a commercial plot exploration; the rest were previously collected materials (Table 1).

For each line, 20 plants were established, their planting framework was 3 m between rows and 2 m between plants. The established plants had basic agronomic management for this crop, consisting of supplying drip irrigation between 2 to 4 h daily; manual and chemical weed elimination, monitoring and chemical managing pests and diseases, and fertilization management with N-P-K nutrient solutions (Coria *et al.*, 2017); likewise, the plants developed with the local environmental conditions (Figure 1).

Table 1. Outstanding papaya lines evaluated in the experimental field.

Line No.	Registry No.	Nomenclature	Line No.	Registry No.	Nomenclature
1	10	H. Barocio 3, Antúnez, P1	13	4	H. Pista, Antúnez
2	9	H. Barocio, 2 Antúnez	14	IX	AR
3	11	H. Barocio 3, Antúnez, P2	15	XI	P5
4	14	H. Andrade, Antúnez	16	III	25A
5	13	H. Andrade, La Soledad	17	IV	42A
6	8	H. Barocio 1, Antúnez P3	18	II	21A
7	7	H. Barocio 1, Antúnez, P2	19	VIII	ARTM
8	6	H. Barocio 1, Antúnez, P1	20	X	P4
9	5	H. Adelo, Ceñidor P3	21	I	9A
10	12	H. Barocio 4, Antúnez	22	2	H. Adelo, Ceñidor, P1
11	III	42 A	23	1	H. Ramón, Antúnez
12	3	H. Adelo, Ceñidor P2	-	-	-

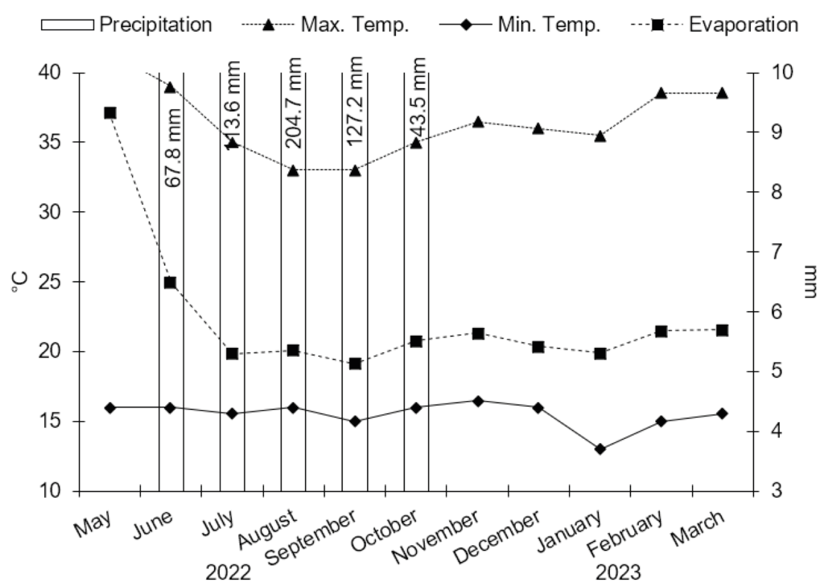


Figure 1. Climatic variation during the essay (Department of Hydrometry, Irrigation District 097, CONAGUA, Mexico).

From the beginning, 23 lines (treatments) and five plants (repetitions) were formed, in a randomized complete block experimental design. In two periods, 94 and 164 days after transplanting (dat), the following was recorded: plant height, using a flexometer, measured from the base of the soil to the plant apex; stem circumference, assessed approximately 15 cm above the soil base using a measuring tape. The number of leaves, visually recorded; and the height of the first fruit, recorded at 94 days, measuring with a flexometer the length between the ground and the first fruit.

During plant development, reviews were conducted to identify outstanding ones. This characteristic consisted of plants with a healthy visual appearance and excellent vigor.

Once they presented their flower buds, hermaphrodite plants were chosen, preferably with heights at the first flower below 0.8 m and fruit precocity. This allowed discarding plants from lines that did not meet the characteristics to be considered outstanding plants. Further attention was directed to the selected plants, where some lines presented one plant. Plants that met these characteristics were differentiated with a plastic signal coiled on the stem. At anthesis, flower buds were chosen from between four and seven fully developed buds and labelled with basic identification information. The flower buds were protected with 4.5×7.5 cm waxed glassine paper bags to ensure self-pollination. During this stage, the number of covered buds, formed fruits, and quality fruits were recorded, that is, morphologically normal fruits for subsequent seed collection. The development of the fruits until their physiological maturity lasted approximately four months. In a complete randomized blocks experimental design, 10 treatments were formed (only the outstanding lines) and five repetitions (fruits). The recorded fruit variables were: polar and equatorial circumference, with a measuring tape, where the fruits were surrounded by two crossing axes; fruit shape index, assessed by dividing the polar with the equatorial circumference of the fruits; fruit weight, weighed with a digital scale; pulp width, slices were cut, and with a graduated ruler, the middle part and mesocarp width were measured; pulp firmness, on a fruit side, the epicarp was removed and the mesocarp pressed with a penetrometer to record its hardness; and soluble solids, juice was extracted from the mesocarp onto a refractometer.

The data analysis of the recorded variables depended on the test. On variables under experimental design, analysis of variance, and comparison of means were performed with the Tukey statistical test ($P=0.05$). Progress of floral bud development and transition to formed fruits and collected fruits, the numerical values were percentagewise compared. All variables were concentrated to perform a multivariate principal components and clusters analysis. Also, the basic statistical indicators of the variables under study were compared. The SAS version 9.3 (2002) and PAST 3.2 (Hammer, 2018) statistical software were used.

RESULTS AND DISCUSSION

Table 2 presents the plant development of the evaluated lines. The analysis of variance showed significant differences in the plant development variables. The four evaluated variables, in their respective samples, expressed variation between lines. Which identifies the morphological characteristics of the lines according to their purpose. The L7 R7 and L15 RXI lines reported higher vigor in the height and stem circumference variables. Regarding the height to the first fruit variable, given that the lowest height materials are the ones required, the L1 R10, L2 R9, L7 R7, L10 R12, and L22 R2 lines meet this condition, as their height to the first fruit did not reach 60 cm. The L3 R11line had the greatest quantity for the number of leaves variable.

As observed in Table 2, the developments of the 23 lines of papaya plants were different during their development stage, which distinguished plant responses to the conditions of the study area. In fact, the national agenda for research, innovation, and agricultural technology transfer suggests these research initiatives and emphasizes that the used materials must be developed for each region of interest (SNITT-SAGARPA, 2016).

Table 2. Development of papaya plants of 23 lines in two sampling periods.

Id.	Plant height (cm)		Stem circumference (cm)		Leaf number		Height of 1st fruit (cm)
	94	164	94	164	94	164	
L1 R10	44.4 hij	84.2 h	10.68 ef	24.49 e	17.2 i	20 f	58 ef
L2 R9	56.4 efgh	110.8 efg	13.82 bcde	35.16 abcd	22.2 abcdef	27.6 bcdef	55 f
L3 R 11	63.8 bcdefg	127.2 cdef	14.01 bcde	34.54 abcd	25.2 a	42 a	71.2 def
L4 R14	49.6 fghi	129.2 bcdef	12.56 efd	33.91 abcd	21.4 cdefgh	31.6 bcde	68.8 def
L5 R13	59.8 bcdefg	128.6 cdef	14.13 bcde	34.54 abcd	22.8 abcde	31.4 bcde	71.2 def
L6 R8	49.2 fghi	131.4 bcde	12.56 def	35.79 abc	20.2 efghi	33.4 bcd	64.8 ef
L7 R7	64.6 bcdefg	140 bc	16.02 abcd	33.91 abcd	24.4 abc	31.6 bcde	53 f
L8 R6	68.2 bcde	142.6 bc	16.96 abc	36.42 ab	23.8 abcd	35.4 abc	69 def
L9 R5	68.2 bcde	133.2 bcd	16.96 abc	32.02 abcd	23.8 abcd	32 bcde	93.6 bcd
L10 R12	64.4 bcdefg	143 bc	18.53 a	37.05 a	23.6 abcd	31 bcde	56.2 f
L11 RIII	48 ghi	113.6 defg	14.13 bcde	30.14 cde	20.8 defgh	32.8 bcde	74.8 cdef
L12 R3	59 defgh	130.2 bcdef	13.50 cde	35.16 abcd	19.2 fghi	36.6 ab	85.6 bcde
L13 R4	74.2 ab	134.2 bcd	17.59 ab	33.28 abcd	24.4 abc	30.4 bcde	100.6 bc
L14 RIX	41.8 ij	114.6 defg	9.67 f	32.65 abcd	25 ab	27 def	73.6 cdef
L15 RXI	82.8 a	194.8 a	17.59 ab	37.05 a	19 fghi	26.6 def	134.4 a
L16 RIII	62.8 bcdefg	149.8 b	15.39 abcd	30.2 cde	20.8 defgh	25 ef	104.4 b
L17 RIV	68.6 bcd	129.6 bcdef	15.70 abcd	33.91 abcd	23.2 abcde	28.6 bcde	93.6 bcd
L18 RII	55.6 fghi	101.6 gh	14.13 bcde	29.51 de	23.2 abcde	26.8 def	64.6 ef
L19 RVIII	36.2 j	94.8 gh	10.68 ef	30.77 bcd	18.6 ghi	26.2 def	60.6 ef
L20 RX	71.6 abc	143.8 bc	14.45 bcde	32.65 abcd	20.6 defgh	27 def	100.6 bc
L21 RI	40.4 ij	110.4 fg	10.68 ef	32.65 abcd	18.2 hi	28 bcdef	67.2 def
L22 R2	54.2 efgh	112 efg	14.13 bcde	32.65 abcd	21.8 bcdefg	27.2 cdef	51.4 f
L23 R1	67 bcdef	111 efg	16.33 abcd	31.4 abcd	20.6 defgh	27 def	67 def
P	**	**	**	**	**	**	**
DMS	11.80	20.62	3.38	5.96	3.32	8.29	28.11
CV (%)	8.51	6.90	11.29	7.65	6.49	11.81	15.75

Means with equal letters are not statistically different (Tukey, 0.05), **: $P \leq 0.001$, MSD: Minimum significant difference, CV: coefficient of variation.

Under this assessment, some evaluated lines showed marked advantages over the rest. According to SNICS-SAGARPA (2014), plants for multiplication purposes must have some characteristics to guarantee quality, such as being vigorous, from varieties with uniform plants, both in size and fruit shape, in addition to low height, pests and disease tolerant, among others. However, for the crop as a whole to achieve high yields and fruit quality, several factors are involved, such as the genetic constitution of the cultivar, favourable soil-climatic conditions, efficient phytosanitary control, timely water supply, and nutritional deficiency corrections (Santana *et al.*, 2018).

Derived from the plants periodic evaluation, only 10 lines reported outstanding characteristics plants. Because the flower buds in these plants were covered, the three-stage flower buds, formed fruits and collected fruits quality process was followed. As observed in Figure 2, the number of formed fruits was reduced in percentage terms compared to the covered buds, as well as the number of fruits collected was reduced in relation to the formed fruits, due to natural factors influencing the process. Thus, the L7 R7 line reached 100% formed fruits in the covered buds, that is, from four covered buds, four fruits were formed. However, in collected fruits quality terms the formed fruits reached only 50%, That is, from four formed fruits, only two quality fruits were collected for seed extraction. In the L19 RVIII line case, the quality collected fruits from the formed fruits was 100%, that is, from three formed fruits, three quality fruits were collected. The same did not happen in the fruits formed from the covered buds at the beginning, since, out of seven covered buds, only three fruits were formed.

Carica papaya is generally propagated by seeds, therefore, high plant heterogeneity is common (Bhattacharya and Khuspe, 2001). The genetic base is limited and depends on a few alternative varieties and hybrids that do not satisfy their demand. This encourages producers to select F2 to F4 generations in continuous plantations, which is why flower bud protection is common practice (Stice *et al.*, 2016), so as not to run the risk of loss of vigor and segregation (Marin *et al.*, 2006). For this reason, the fundamental principle of having a broad genetic base is pursued to choose promising materials, where plant selection is a good beginning for crop improvement. Meanwhile, of 23 promising lines, only 10 were selected, the rest were purged.

The variance analyses on the fruit characterization variables are shown in Table 3. Except for the soluble solids variable, all the variables had significant differences. The polar and equatorial circumference in the L14 RIX line reported the largest fruit dimensions, opposite values were recorded in line L7 R7. For the shape index variable, line L13 R4 had the highest proportion. At the same time, the L19 RVIII line had the lowest shape index. Regarding fruit weight, the L21 RI line had the heaviest fruits, the opposite situation occurred in the fruits of the L7 R7 line, just as they had the smallest pulp width, while the L18 RII line had the largest pulp width. In pulp firmness, the L21 RI line presented the highest pulp hardness.

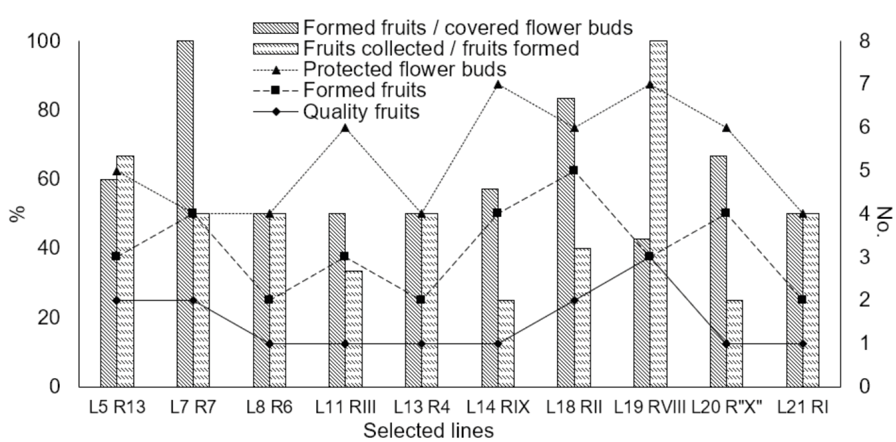


Figure 2. Development process between stages from flower buds to formed fruits of papaya.

Table 3. Characterization of fruits collected from outstanding papaya plants.

Line	Circumference (cm)		Form index	Weight (kg)	Pulp width (cm)	Pulp firmness (kg cm^{-2})	Soluble solids (°Brix)
	Polar	Equatorial					
L5 R13	56.2 abc	31.8 ed	1.76 abc	1.173 bc	2.74 bcd	2.14 ab	12.52
L7 R7	51 c	28.6 e	1.79 ab	0.822 c	2.42 d	2.04 b	12.78
L8 R6	56.8 abc	34.6 bcd	1.64 bcd	1.239 bc	2.66 cd	2.2 ab	12.42
L11 RIII	62.4 a	27.2 abc	1.67 abcd	1.722 abc	3.1 abc	2.14 ab	12.34
L13 R4	61.2 ab	32.2 cde	1.89 a	1.297 bc	2.66 cd	2.2 ab	12.68
L14 RIX	62.2 a	40.2 a	1.54 cd	1.73 abc	3.02 abc	2.08 b	12.38
L18 RII	61 ab	38.4 ab	1.58 bcd	1.806 ab	3.22 a	2.06 b	12.86
L19 RVIII	59.2 abc	38.8 ab	1.52 d	1.721 abc	3.16 ab	2.14 ab	12.6
L20 R"X"	53 bc	31 de	1.71 abcd	0.95 bc	2.74 bcd	2.08 b	12.16
L21 RI	61.8 a	38.8 ab	1.58 bcd	2.51 a	3.08 abc	2.32 a	12.1
P	**	**	**	**	**	**	ns
DMS	8.54	5.29	0.22	0.92	0.47	0.21	1.07
CV (%)	6.85	7.06	6.26	28.95	7.78	4.77	4.03

Means with equal letters are not statistically different (Tukey, 0.05), **: P≤0.001, ns: not significant, MSD: minimum significant difference, CV: coefficient of variation.

Fruit characteristics are commonly important variables that allow genotype choosing (Oliveira de *et al.*, 2012). The fruit weight was acceptable, ranging between 0.822 to 1.806 kg, this in turn was reflected in the fruit size, shape index, and pulp width, whose trend was similar. Both soluble solids and pulp hardness express fruit quality. These characteristics correspond to the 'Maradol' variety. Furthermore, under the proposed scheme, the self-pollinated flowers of hermaphrodite plants, according to the floral proportion in papaya, expected 66.67% hermaphrodite offspring plants for their next cycle (Santana *et al.*, 2019).

The multivariate analysis of the 12 considered variables was interpreted based on the eigenvalues where the individual and accumulated variance in each component of the analysis is shown. Likewise, the eigenvalue and variance of the correlation matrix. The shown values indicate the associated variability with each principal component, and it reduces as it increases the components number, cumulatively showing that the first component explains 68% of the variability (Table 4).

Table 4. Eigenvectors of plant and fruit characteristics, and variability proportion of variance in outstanding papaya plants.

Principal component	Variance-covariance matrix			Correlation matrix	
	Proper value	Explained variance (%)	Cumulative variance (%)	Proper value	Variance (%)
1	845.614192	0.6886	0.6886	845.614	68.858
2	336.012629	0.2736	0.9622	336.013	27.362
3	31.456645	0.0256	0.9878	31.4566	2.5615
4	8.823233	0.0072	0.9950	8.82323	0.71848
5	4.413165	0.0036	0.9986	4.411316	0.35936
6	1.343405	0.0011	0.9997	1.34341	0.10939

Likewise, the first two principal components explained the accumulated variability in the selected lines (Figure 3). Since the first component exceeds 68% of all variance it is positively correlated with plant height (Table 5). The second component represents 24% of all the variance and is positively correlated with the yield per plant (Table 5).

For its part, with the structures of the two principal components, a cluster analysis was performed. This analysis considered the structure of the first two principal components since they explained 96% of the variance and could facilitate identifying variant groups. The results showed that at a Euclidean distance of 20, three defined groups were formed: L11 RIII, L19 RVI, and L13 R4; L18 RII and L5 R13; L8 R6 and L20 RX. On the other hand, L14 RIX, L21 RI, and L7 R7 were not part of any group (Figure 4).

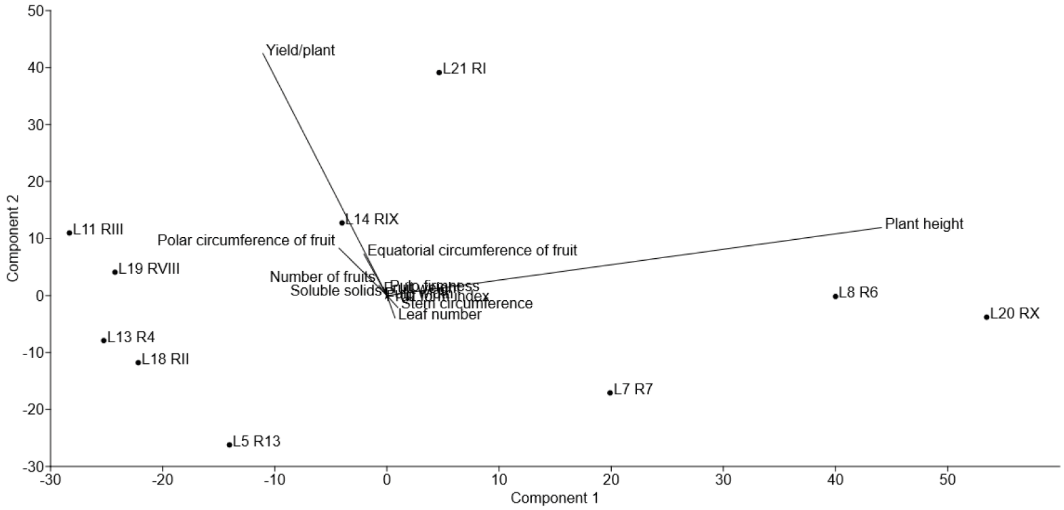


Figure 3. Diagrammatic dispersion of selected lines and variables of plants and fruits in principal components 1 and 2.

Table 5. Eigenvalues of principal components 1 and 2 in the recorded variables.

Variable	Principal component 1	Principal component 2
Polar circumference of fruit	−0.0934426	0.18166
Equatorial circumference of fruit	−0.044811	0.15857
Fruit form index	−0.0014479	−0.0021231
Fruit weight	−0.0064373	0.030484
Pulp width	−0.0040511	0.009336
Pulp firmness	−0.00063862	0.0038579
Soluble solids	−0.0029458	−0.012002
Plant height	0.96428	0.26089
Stem circumference	0.021186	−0.045933
Leaf number	0.01583	−0.085808
Number of fruits	−0.015518	0.038721
Yield / plant	−0.24171	0.92824

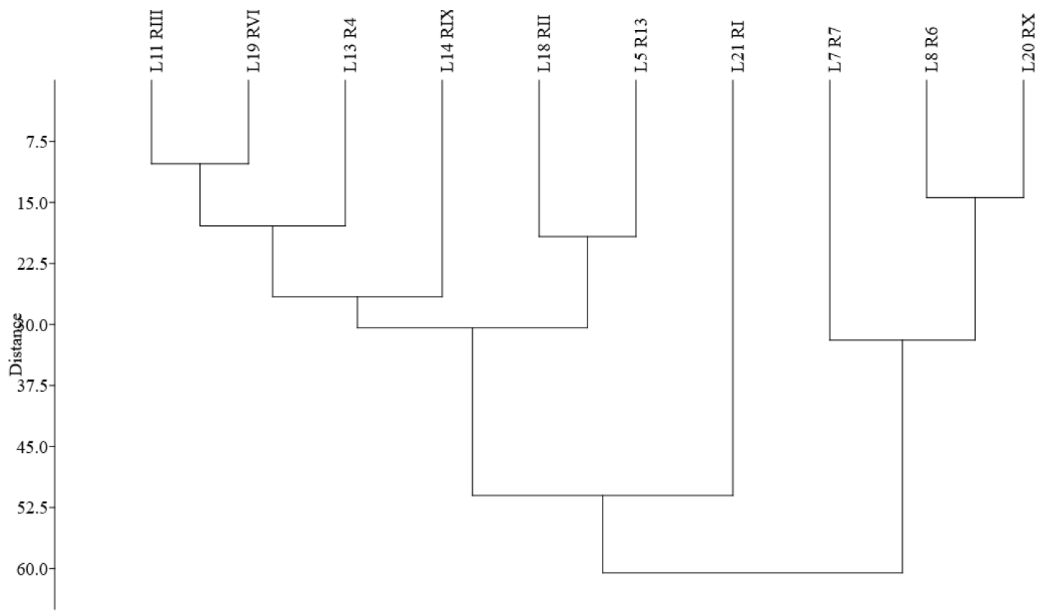


Figure 4. Dendrogram of 12 variables of plants and fruits of selected lines, with three defined groups and three undefined groups at a Euclidean distance of 20.

The principal components analysis required two components to explain 96% of all the variance, the first component contributed 68% of the total variance. With this information, three groups were defined through the cluster analysis. Aikpokpodions (2012) evaluated 60 papaya materials using 21 variables defined from descriptors. Their multivariate analysis generated five groups, thus revealing a significant variation that can be used for the papaya genetic improvement. For their part, Saran *et al.* (2015) evaluated 24 papaya materials and 29 morphological characteristics. Their multivariate analysis showed high morphological diversity in fruit yield, weight, length, cavity, fruiting zone, pulp thickness, pulp color, and soluble solids, whose response is similar to that reported here. Therefore, exploratory studies are important to identify promising materials, for the implementation of a strategy for multiplication, distribution, and improvement of the crop. In fact, the study of genetic diversity is essential in the preliminary selection of accessions with superior characteristics and for the successful use of these genotypes in breeding programs (Barbosa *et al.*, 2011).

Regarding the sample statistics derived from values calculated in variables recorded from papaya plants, these are shown in Table 6. The recorded values did not deviate from normality.

Given that the strategy is to increase productivity in a sustainable and balanced way, searching for new genotypes is crucial for performance improvement (Nascimento *et al.*, 2019). In germplasm collections, genetic diversity allows the assessment of qualitative or quantitative morphology, whose focus is on the evaluation of population segregation by genetic parameters estimation, using selection indices and estimates of correlations between traits related to yield and quality of fruit are necessary (Barbosa *et al.*, 2011).

Table 6. Sample statistics of the variables recorded in outstanding papaya plants.

Variable †	Mean	Standard error	variance	Standard deviation	Minimum value	Maximum value	Coefficient of variation
PCF (cm)	58.20	1.46	21.44	4.63	50.50	63.67	7.96
ECF (cm)	34.80	1.20	14.43	3.80	29.75	40.00	10.91
FFI	1.71	0.04	0.02	0.13	1.58	1.93	7.78
PW (kg)	1.52	0.21	0.44	0.66	0.93	3.15	43.36
PF (cm)	2.87	0.09	0.08	0.28	2.40	3.20	9.79
FP (kg cm^{-2})	2.14	0.03	0.01	0.10	2.00	2.33	4.47
SS (°Brix)	12.49	0.10	0.09	0.31	11.93	13.00	2.46
PH (cm)	222.40	9.00	809.16	28.45	196.00	273.00	12.79
SC (cm)	47.77	0.93	8.61	2.93	41.41	52.08	6.14
LN	42.63	0.74	5.50	2.34	38.88	47.00	5.50
NF	32.80	1.70	29.07	5.39	24.00	40.00	16.44
Y/P (kg)	49.04	5.82	339.22	18.42	28.49	85.02	37.56

† PCF=polar circumference of fruit; ECF=equatorial circumference of fruit; FFI=fruit form index; FW=fruit weight; PW=pulp width; PF=pulp firmness; SS=soluble solids; PH=plant height; SC=stem circumference; LN=leaf number; NF=number of fruits; Y/P=Yield/plant.

CONCLUSIONS

The plant morphological variables recorded values resemble the characteristics of the 'Maradol' type, whose variability allowed the identification of prospective materials. Of the 23 evaluated papaya lines, only 43.48% presented outstanding plants, so 13 lines were discarded. Within the selected lines, only between 5 and 10% of the plants were chosen for their outstanding characteristics. In the development progress from the covered buds stage to formed and harvested fruits, the average value of the 10 lines gradually reduced, in 60% of the formed fruits stage over the covered buds stage and 47% of the fruits stage collected during the stage of formed fruits. Overall, only 28% of the collected fruits on the covered buds were rescued. The selected lines showed variability in fruit values, where the shape index, fruit weight, and pulp width were important indicators to define a mode preference. The multivariate analysis classified three defined groups.

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Colegio de Postgraduados

Worm castings and naphthaleneacetic acid in the growth and yield of zucchini in shade houses

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ABSTRACT
The cultivation of zucchini (*Cucurbita pepo* L.) under protected conditions has limitations, such as low fruit set due to poor pollination, which affects the commercial yield and crop profitability. This research was carried out to assess the influence of earthworm humus (HL) and naphthaleneacetic acid (ANA) on the growth and yield of two zucchini cultivars in shade houses. A randomized block experimental design with eight treatments and 10 repetitions was used. The treatments influenced plant height, leaf area index, stem dry weight, leaf dry weight, plant dry weight, and fruit set; However, there were no differences because of the treatments in stem diameter, leaf greenness, and number of leaves. With the HMX58 cultivar, the highest plant height was obtained: while because of the Prestige cultivar, the highest index of leaf area, dry weight of leaves, and dry weight of the whole plant. The highest yield was obtained with the HMX58+LC treatment, while the fruit set and the number of fruits increased because of earthworm humus. The diameter and weight of the fruit increased because of ANA in both cultivars and the length of the fruit in the Prestige cultivar.
Keywords: Cucurbita pepo L.; dry weight; fruit length; fruit set; protected agriculture.

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INTRODUCTION

The utilization of composts and worm castings is a cultural and ecological practice that improves the conditions of agricultural soils (Villegas-Cornelio and Laines, 2016) and induces an increase in the commercial yield of crops (De la Cruz-Lázaro *et al.*, 2009; Blouin *et al.*, 2019).

Besides containing essential nutrients for plants, worm castings have a positive effect on the porosity, aeration, and soil water retention capacity, contributing to the maintenance and development of microflora and microfauna, increasing the availability and root's nutrients assimilation (Aremu *et al.*, 2015; Zanor *et al.*, 2018). They stimulate soil biochemical reactions that promote the emergence, flowering, and fruiting of plants (Ramos *et al.*, 2019; Steffen *et al.*, 2019), since they produce substances with phytohormonal effects (Domínguez *et al.*, 2010), such as indoleacetic acid, gibberellic acid, and kinetin (Ravindran *et al.*, 2016).

Phytohormones present in worm castings play an important role in regulating various plant growth processes (Zhang *et al.*, 2015; Wong *et al.*, 2020). Auxins promote cell elongation, inhibit primary root growth, mediate the response to tropisms, repress organ abscission, and induce floral and fruit development; Gibberellins regulate plant growth, seed germination, stem elongation, reserves mobilization, as well as their floral and fruit development; and cytokinins stimulate cell division, activate bud sprouting, induce organogenesis and delay senescence (Jordán and Casaretto, 2006).

The exogenous application of plant growth regulators depends on several factors such as the plant genotype, growing season, type of regulator, and applied concentration (Tantasawat *et al.*, 2015). Some have been widely used on vegetables, to improve fruit setting and increase production, such as indoleacetic acid (Montaño-Mata and Méndez-Natera, 2009), gibberellic acid (Pichardo-González *et al.*, 2018), naphthaleneacetic acid (Soriano-Melgar *et al.*, 2020; Sedighehsadat *et al.*, 2021), β-naphthoxyacetic acid (Martínez *et al.*, 2016) and forchlorfenuron (CPPU) (Rueda-Luna *et al.*, 2018). In this sense, naphthaleneacetic acid has been widely used in melons (Barzegar *et al.*, 2015; Menchaca-Ceja *et al.*, 2018), however, its effect on zucchini is scarcely known.

Regarding cucurbit cultivation in Mexico, zucchini (*Cucurbita pepo*) occupies the third place in economic importance (3,386 million pesos, MXN), preceded by the cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*). The states of Sonora and Puebla occupy first and second place in cultivated zucchini areas, with 6,200 and 3,889 ha, respectively. The third, Sinaloa has a 2,426 ha planted area, second place in production (78,215 t), and first place in yield (32.28 t ha^{-1}), 40% above the national average (SIAP, 2021).

However, staking cucurbit cultivation in protected conditions, regardless of using specific techniques to improve zucchini production, is scarcely researched in Mexico. Therefore, the objective of this research was to evaluate the effect of applying worm castings to soil and naphthaleneacetic acid via foliar spraying on the growth and yield of zucchini under shaded housing conditions.

MATERIALS AND METHODS

The experiment took place within a crop protection structure, shade house type, at the experimental field of the Facultad de Agronomía of the Universidad Autónoma de Sinaloa, located at 24° 48' 30" NL, 107° 24' 30" WL, 38.54 m altitude. The soil analysis, carried out before cultivation indicated that the soil had a clay texture and low organic matter content (Table 1). Culiacán, Sinaloa, has a BS1(h')w(w)(e) climate: very warm semi-dry, extreme with summer rains, with less than five percent partial winter precipitation regarding the annual total (García, 2004). The mean temperature (21.9 °C) and relative humidity (69.5%) were recorded using thermohygrometers (DT171, Twilight) during the crop cycle (11/16/2019 to 03/04/2020), these were within optimal ranges (18 to 24 °C and 65 to 80% relative humidity) for zucchini cultivation (Molinar *et al.*, 1999; Cortés, 2003).

The soil was prepared and beds were formed 1.8 m apart from each other, to half of which worm castings were applied (Table 1). Subsequently, double irrigation tape and white/black coextruded polyethylene mulch were placed on top of each bed. Sowing was

Table 1. Physicochemical and microbiological characteristics of the soil and worm castings.

Analysis		Floor	Earthworm humus
Physical parameters:	pH 1:1 (H_2O)	7.85	5.98
	CE (dS m^{-1})	0.58	7.75
	PS (%)	79	96
	MO (%) Walkley-Black	1.21	26.49
	Sand (%)	21	77
	Silt (%)	20	16
	Clay (%)	59	7
Cations: (cmol+ kg^{-1})	Na^+ (Ac. NH4 pH 7)	2.2	0.91
	K^+ (Ac. NH4 pH 7)	0.22	2.11
	Ca^{2+} (Ac. NH4 pH 7)	2.32	43.16
	Mg^{2+} (Ac. NH4 pH 7)	0.78	13.57
Anions: (mg kg^{-1})	N-NO_3^- (Brusina)	0.97	3.4
	P-PO_4^- (Bray I)	0.06	0.29
	S-SO_4^{2-} (Turbidimeter)	0.12	50.43
Phytobeneficial: (ufc g^{-1} o propágulos g^{-1})	Aerobic bacteria (BK)	266 667	3 400 000
	Anaerobic bacteria (BK)	116 667	913 333
	Bacillus sp. (BK)	243 333	1 666 667
	Actinomycetes (AN)	100 000	233 333

conducted in 128-cavity polystyrene trays filled with peat (Brown 025W, Kekkila). The transplant took place on November 16, 2019, in an 11,820 plants per hectare density.

The Steiner (1984) solution at 50% was supplied for crop nutrition, from the transplant until the first flower anthesis, the complete solution was added later. Irrigation was applied when the tensiometers (2725ARL, Soil Moisture Equipment) placed at 0.30 m depth of the soil indicated moisture tensions from 20 to 25 kPa.

A randomized block experimental design with a 2A×2B×2C factorial arrangement was used, with eight treatments and 10 repetitions. Factor A corresponded to zucchini: Prestige cultivar, of the "green zucchini" type, and HMX586429 (HMX58), of the "gray zucchini" type. Factor B corresponded to the worm humus (WH) applied to the soil at a rate of 0 and 10 t ha^{-1}. Factor C was naphthaleneacetic acid (ANA), sprayed at 0 and 50. mg L^{-1} doses.

The treatments (interaction A×B×C): Prestige+worm castings+naphthaleneacetic acid (T1=Prestige+WH+ANA), Prestige+worm castings (T2=Prestige+WH), Prestige+naphthaleneacetic acid (T3=Prestige+ANA), Prestige (T4=Prestige), HMX586429+worm castings+naphthaleneacetic acid (T5=HMX58+WH+ANA), HMX586429+worm castings (T6=HMX58+WH), HMX586429+naphthaleneacetic acid (T7=HMX58+ANA) and HMX586429 (T8=HMX58) were established with ten repetitions in a 250 m^2 experimental area, with three plants as a useful plot.

The evaluated growth variables were: plant height (PH), with a measuring tape; stem diameter (SD), using a digital vernier (6MP, Truper); number of leaves per plant (LN); foliar greenness index (FG), with a portable chlorophyll meter (SPAD 502, Minolta), leaf area index (LIA) in leaf area m^2 per soil surface m^2, obtained by dividing the foliar area per each plant occupied surface (0.81 m^2), each leaf foliar area was estimated with the equation:

$$FA = 4.77 + \left(0.61 \times \text{width}^2\right),$$

following Rouphael *et al.* (2006). The stem dry weight (SDW), leaves (LDW), and plant (PDW), obtained at the end of the crop cycle (109 days after transplanting), were also evaluated with a precision balance (CP622, Sartorius), after drying the plant material in an electric oven (292, Felisa) at 70 °C until constant weight.

Regarding zucchini production, the fruits were collected between 5 and 7 days after anthesis and the number of fruits (NF) per plant was counted, with which the following was determined:

$$\text{Percentage of fruit setting}\left[FS(\%) = \left(\frac{\text{number of fruits with length } \geq \text{ 127 mm}}{\text{number of female flowers}}\right) \times 100\right];$$

fruit diameter (FD) and length (FL), using a digital vernier (6MP, Truper); fruit weight (FW), with a precision scale (CP622, Sartorius), yield (Molinar *et al.*, 1999; USDA, 2016) and fruit lag.

The obtained data were assessed with an analysis of variance and Tukey's multiple means comparisons test ($p \leq 0.05$) using version 7.0 STATISTICA statistical analysis software (StatSoft, 2004).

RESULTS AND DISCUSSION

Plant height was only affected by the cultivar factor (Table 2) since HMX58 exceeded ($p \leq 0.05$) the plant height of the Prestige cultivar by 53.6%. This obtained plant height concurs with Román-Román *et al.* (2022), who also observed greater height in HMX58 cultivar plants, compared to those of the Prestige cultivar. Dasgan and Bozkoylu (2007), Díaz *et al.* (2016) and Ayala-Tafoya *et al.* (2020) recorded heights of 210.9 to 288.3, 76, and 53.2 cm, under greenhouse environments, in 'Alata Yesili', 'Spineless Perfection' and 'Obsession' pumpkin plants, respectively. All of this coincides with Loy (2004), who points out the considerable morpho-physiological variation between pumpkins (*Cucurbita* spp.) and even between same species cultivars.

The cultivar factor also significantly influenced ($p \leq 0.05$) the stem diameter and leaf greenness (SPAD); although in both cases, without statistical differences ($p \leq 0.05$) due to the effect of the treatments. The stem diameter and leaf greenness index, observed during the evaluation period, indicate adequate plant vigor (Mendoza *et al.*, 1998; Swiader and Moore 2002; García-Bañuelos *et al.*, 2016) and concur with that reported on both variables by Ayala-Tafoya *et al.* (2020) and Román-Román *et al.* (2022).

Likewise, neither the number of produced leaves per plant (62.9 to 67) during the evaluation period nor the number of leaves (LN) that remained on the plants (22.7 to 26.3), after five pruning (de-leafing), were significantly affected by the treatments (Table 2). To avoid photosynthates loss in lower senescent leaves, reduce *Oidium* sp inoculum and the immature *Bemisia tabaci* population (Román-Román *et al.*, 2022), four to six leaves/plant were pruned every 12 to 14 days, thereby maintaining a more or less constant leaf density from 40 days after transplanting (dat) on, which is similar to the number of leaves (22 to 26) reported by Monares-Gallardo *et al.* (2012) and Román-Román *et al.* (2022).

The leaf area index was significantly influenced by the cultivar and naphthaleneacetic acid factors, as well as by the cultivar × WH interaction. The highest leaf area index was obtained with the Prestige treatment plants, statistically equal to the response induced by Prestige+WH, HMX58+WH, and Prestige+ANA, which exceeded from 41.2 (HMX58+ANA) to 118.2% (HMX58) that obtained in the rest of the treatments (Table 2). The leaf area index obtained with 'Prestige' is similar to that reported by Rouphael and Colla (2005) in 'Aphrodite' zucchini plants in greenhouse conditions (LAI of 2.7 m^2 m^{-2}) and Román-Román *et al.* (2022) with the same cultivar. The observed differences in the leaf area index also concur with Loy (2004), who reports considerable morpho-physiological variation between *Cucurbita pepo* cultivars.

Table 2. Effect of worm castings (WH) and naphthalencetic acid (ANA) on height (PH), stem diameter (SD), leaf greenness (FG), leaf number (LN), and leaf area index (LAI).) of plants of two zucchini cultivars under shaded housing conditions.

Treatment	AP (cm)	DT (mm)	VF (SPAD)	NH	IAF (m^2 m^{-2})
T1=Prestige+HL+ANA	92.1 b[§]	14.6 a	52.8 a	23.9 a	1.5 bc
T2=Prestige+HL	91.9 b	15.0 a	52.7 a	24.4 a	2.0 ab
T3=Prestige+ANA	91.9 b	14.8 a	54.4 a	24.3 a	1.8 ab
T4=Prestige	93.1 b	14.7 a	52.2 a	26.3 a	2.4 a
T5=HMX58+HL+ANA	139.0 a	13.4 a	49.8 a	22.7 a	1.5 bc
T6=HMX58+HL	147.8 a	13.8 a	50.6 a	26.0 a	2.0 ab
T7=HMX58+ANA	143.3 a	13.6 a	50.1 a	25.1 a	1.1 c
T8=HMX58	136.5 a	14.0 a	52.1 a	24.1 a	1.7 bc
DMSH	20.6	2.8	7.3	4.7	0.6
Significance					
CULTIVATE	***	*	*	ns	**
HL	ns	ns	ns	ns	ns
ANA	ns	ns	ns	ns	***
CULTIVATE×HL	ns	ns	ns	ns	**
CULTIVATE×ANA	ns	ns	ns	ns	ns
HL×ANA	ns	ns	ns	ns	ns

[§] Means with equal letters are not statistically different (Tukey≤0.05). DMSH=honest least significant difference; ns, *, **, ***: not significant at $p \leq 0.05$, significant at $p \leq 0.05$, $p \leq 0.01$ y $p \leq 0.001$.

The leaf dry weight was significantly influenced by the cultivar and naphthaleneacetic acid factors, as well as the interaction of these (Table 3). The Prestige cultivar plants exceeded the dry weight of leaves of the HMX58 cultivar plants by 51.6%. The highest dry weight of leaves was obtained in the Prestige treatment, without differences with the Prestige+WH, but higher than 53.3 (Prestige+ANA) to 115.2% (HMX58+ANA) than the dry weight of leaves induced by the rest of the treatments. Also, due to the naphthaleneacetic acid application, the leaves dry weight decreased. The cultivar factor affected the stem dry weight since this variable of the HMX58 cultivar exceeded that of the Prestige cultivar by 22.1% (Table 3). The highest stem dry weight was obtained with HMX58+LC, which exceeded that produced by Prestige+ANA and Prestige+WH+ANA by 49.6 and 51.2%, but with no differences with the effect of the rest of the treatments.

The cultivar and naphthaleneacetic acid factors affected the plant's dry weight; since, the Prestige cultivar exceeded that variable by 17.7% compared to the HMX58 cultivar, and with the naphthaleneacetic acid application the dry weight per plant decreased by 14.5%. (Table 3). Thus, the Prestige treatment plants had the highest dry weight, although it only exceeded that obtained in the HMX58+ANA, HMX58, and HMX58+WH+ANA treatments, by 31.5 to 39.1%.

Table 3. Effect of worm castings (WH) and naphthaleneacetic acid (ANA) on leaves dry (LDW), stem (SDW), and whole plant (PDW) weight of two zucchini cultivars under shaded housing conditions.

Treatment	PSH (g)	PST (g)	PSP (g)
T1=Prestige+HL+ANA	132.6 bc[§]	45.7 b	364.7 abc
T2=Prestige+HL	175.0 ab	51.1 ab	438.6 ab
T3=Prestige+ANA	135.2 bc	46.2 b	367.1 abc
T4=Prestige	207.2 a	56.3 ab	450.0 a
T5=HMX58+HL+ANA	99.2 c	63.0 ab	342.0 bc
T6=HMX58+HL	130.3 bc	69.1 a	387.1 abc
T7=HMX58+ANA	96.8 c	57.5 ab	323.5 c
T8=HMX58	102.5 c	53.9 ab	324.1 c
DMSH	59.2	21.3	108.4
Significance			
CULTIVATE	***	**	***
HL	ns	ns	ns
ANA	***	ns	**
CULTIVATE×HL	ns	ns	ns
CULTIVATE×ANA	*	ns	ns
HL×ANA	ns	ns	ns

[§] Means with equal letters are not statistically different (Tukey≤0.05). DMSH=honest least significant difference; ns, *, **, ***: not significant at p≤0.05, significant at p≤0.05, p≤0.01 y p≤0.001.

The observed differences in dry weight production coincide with the morpho-physiological variation between zucchini cultivars reported by Loy (2004). Furthermore, the dominant force on the photosynthates demand exerted by the fruits, related to the production capacity assimilated by the plant, can also regulate vegetative development (Loy, 2004; Sedano-Castro *et al.*, 2005; Luna *et al.*, 2015; Orozco *et al.*, 2016). This explains the greater dry weight in the Prestige cultivar plants, compared to those from the HMX58 cultivar, which developed more fruits per plant (Table 4).

The fruit set was affected by the cultivar and worm castings factors since the plants of the Prestige cultivar exceeded the fruit set of HMX58 by 34.8%, with the vermicompost application, this increased by 22.6%. The interaction between cultivar and worm castings, cultivar and naphthaleneacetic acid, or worm castings and naphthaleneacetic acid also influenced this variable (Table 4). A higher percentage of fruit set was obtained with the Prestige+WH treatment, which exceeded that achieved with HMX58+ANA, HMX58, and HMX58+WH+ANA, from 42.3 to 199.6%. The fruit set percentage obtained with pumpkin plants managed without pollination (24.8 to 68.3%), concurs with the 38 to 82% reported by Robinson and Reiners (1999), who point out that some cultivars' ability to produce fruits without pollination is fortuitous because they were not selected for that characteristic. They also agree with Kurtar (2003), who obtained the highest fruit set percentages in "green zucchini" type cultivars compared to other types of zucchini. Overall, the obtained fruit setting result is close to the 49.3% fruit setting reported by Pagoto *et al.* (2020) with 'Antonella' zucchini and is also in the range of 59.5 to 77.9% fruit set of 'Obsession' zucchini reported by Ayala-Tafoya *et al.* (2020), or the 56% fruit set of 'Tosca' squash without pollination described by Knapp and Osborne (2017).

The increase in fruit set achieved with the application of worm castings shows that its soil application as a source of natural growth-promoting substances (Fritz *et al.*, 2012; Calvo *et al.*, 2014; Aremu *et al.*, 2015), can be useful to increase the concentrations of endogenous phytohormones and promote zucchini fruit's growth (Li *et al.*, 2002; 2005), in the absence of pollinating insects.

The cultivar, worm castings, and naphthaleneacetic acid factors, and the interaction between worm castings and naphthaleneacetic acid influenced the number of fruits produced per plant (Table 4). The HMX58 cultivar produced 15.1% more fruits than the Prestige cultivar, while worm castings application increased this variable by 10.7%. The HMX58+WH treatment induced the highest fruits per plant number, with no difference with the Prestige+WH response, which exceeded that induced by the rest of the treatments, from 18.4 (HMX58) to 55.6% (Prestige+WH+ANA). The number of fruits per plant is similar to research by Rodríguez-Dimas *et al.* (2007) and Ayala-Tafoya *et al.* (2020), who report an increased number of fruits per plant as a consequence of soil application of worm castings; from this practice, Mogollón *et al.* (2014) report increases in enzymatic activity and edaphic respiration.

The monoesy of cucurbit plants requires the interaction with pollinating insects to achieve fruit set and production (Vidal *et al.*, 2010; Petersen *et al.*, 2013), or performing manual pollination to optimize the fruiting under conditions of mesh housing when bees or bumblebees are not available (Román-Román *et al.*, 2022). However, Ayala-Tafoya *et* *al.* (2020) report that vermicompost application in zucchini cultivation induced fruit set. Additionally, applications of organic fertilizer also promote an increase in the diameter, length, and weight of fruit variables (Rodríguez-Dimas *et al.*, 2007; Ayala-Tafoya *et al.*, 2020).

Fruit diameter was significantly influenced by all the main analyzed factors and interactions (Table 4). The HMX58 cultivar reported a fruit diameter 15.7% larger than that observed in the Prestige cultivar. The worm castings and naphthaleneacetic acid application increased it by 6.4 and 9.9%, respectively. Therefore, the HMX58+WH, HMX58+ANA, and HMX58+WH+ANA treatments induced a fruit diameter that exceeded the effect of the other treatments, from 9.5 (Prestige+ANA) to 38.9% (Prestige).

All factors and interactions also influenced the fruit length variable, except for the cultivar and worm castings interaction (Table 4). However, contrary to the fruit diameter, a 30.9% greater fruit length was recorded in the Prestige cultivar compared to the HMX58. Likewise, with the worm castings and naphthaleneacetic acid application, fruit length increased by 6.1 and 10.7%, respectively. The Prestige+ANA and Prestige+WH+ANA treatments had a fruit length that exceeded the effect caused by the other treatments, between 9 (Prestige+LC) to 56.7% (HMX58).

Fruit weight was favored by the worm castings and naphthaleneacetic acid factors, and by the cultivar and naphthaleneacetic acid, and between worm castings and naphthaleneacetic acid interactions (Table 4). The application of worm castings also

Table 4. Effect of worm castings (WH) and naphthaleneacetic acid (ANA) on fruit set (FS), number (NF), diameter (FD), length (FL), and fruit weight (FW) of two zucchini cultivars under shaded housing conditions.

Tratamiento	CF (%)	NF	DF (mm)	LF (mm)	PF (g)
T1=Prestige+HL+ANA	52.1 ab§	26.8 e	37.9 bc	187.4 a	202.6 a
T2=Prestige+HL	68.3 a	36.1 ab	35.2 cd	171.9 b	158.9 bc
T3=Prestige+ANA	58.2 ab	28.4 de	38.8 b	189.0 a	213.0 a
T4=Prestige	57.8 ab	30.2 cde	31.9 d	154.8 c	120.6 c
T5=HMX58+HL+ANA	48.0 b	32.7 bcd	42.5 a	137.8 d	189.0 ab
T6=HMX58+HL	58.4 ab	41.7 a	44.3 a	141.3 cd	195.1 ab
T7=HMX58+ANA	44.2 b	30.2 cde	43.2 a	137.5 d	197.1 ab
T8=HMX58	24.8 c	35.2 bc	36.4 bc	120.6 e	119.7 c
DMSH	15.2	5.4	3.2	13.1	37.1
Significance					
CULTIVATE	***	***	***	***	ns
HL	***	***	***	***	***
ANA	ns	***	***	***	***
CULTIVATE×HL	**	ns	*	ns	ns
CULTIVATE×ANA	*	ns	*	***	*
HL×ANA	***	**	***	***	***

§ Means with equal letters are not statistically different (Tukey≤0.05). DMSH=honest least significant difference; ns, *, **, ***: not significant at p≤0.05, significant at p≤0.05, p≤0.01 y p≤0.001.

promoted an increase in the fruit weight by 14.6%, naphthaleneacetic acid increased it by 34.9%. The highest fruit weight was recorded in the Prestige+ANA and Prestige+WH+ANA treatments, whose average weight exceeded 68 to 77.9% obtained in the Prestige+WH, Prestige, and HMX58 treatments; but, with no differences with the other treatments' effects.

ANA treatments promoted increasing fruit diameter, length, and weight in both cultivars. Auxins promote cell enlargement, as a consequence of the plasticity of cell walls increase (Jankiewics and Acosta-Zamudio, 2003; Ayala-Tafoya *et al.*, 2012), and because the tissues where auxins have a sufficiently high concentration become an attraction point for nutrients and other hormonal substances such as gibberellins (Jankiewics and Acosta-Zamudio, 2003).

The cultivar and worm castings factors, and the interactions between cultivar and worm castings, worm castings, and naphthaleneacetic acid, had a significant effect on the yield (Table 5). The HMX58 cultivar produced 54.2% more yield than the Prestige cultivar, while with vermicompost it increased by 31.3%, compared to the non-application of organic fertilizer. The highest yield was obtained with the HMX58+WH treatment, which exceeded the yield achieved with the rest of the treatments, from 38.1 (HMX58+WH+ANA) to 183.2% (Prestige).

The yield with cultivar HMX58 and with the HMX58+WH treatment is similar to that reported by Román-Román *et al.* (2022) for the same cultivar, with (79.7 t ha^{-1})

Table 5. Effect of worm castings (WH) and naphthaleneacetic acid (ANA) on commercial yield and lagging fruits of two zucchini cultivars under shade housing conditions.

Treatment	CF (%)	DF (mm)	LF (mm)	PF (g)	Yield (t ha^{-1})
T1=Prestige+HL+ANA	52.1 ab[§]	37.9 bc	187.4 a	202.6 a	36.0 cd[§]
T2=Prestige+HL	68.3 a	35.2 cd	171.9 b	158.9 bc	50.0 bc
T3=Prestige+ANA	58.2 ab	38.8 b	189.0 a	213.0 a	44.4 bcd
T4=Prestige	57.8 ab	31.9 d	154.8 c	120.6 c	29.7 d
T5=HMX58+HL+ANA	48.0 b	42.5 a	137.8 d	189.0 ab	60.9 b
T6=HMX58+HL	58.4 ab	44.3 a	141.3 cd	195.1 ab	84.1 a
T7=HMX58+ANA	44.2 b	43.2 a	137.5 d	197.1 ab	60.6 b
T8=HMX58	24.8 c	36.4 bc	120.6 e	119.7 c	41.3 cd
DMSH	15.2	3.2	13.1	37.1	16.1
Significance					
CULTIVATE	***	***	***	ns	***
HL	***	***	***	***	***
ANA	ns	***	***	***	ns
CULTIVATE×HL	**	*	ns	ns	**
CULTIVATE×ANA	*	*	***	*	ns
HL×ANA	***	***	***	***	***

[§] Means with equal letters are not statistically different (Tukey≤0.05). DMSH βhonest least significant difference; ns, *, **, ***: not significant at p≤0.05, significant at p≤0.05, p≤0.01 y p≤0.001.

and without manual pollination (56.7 t ha^{-1}). This indicates that besides the natural parthenocarpy level of the cultivar, the yield was favored by the soil worm castings application, since this, in addition to increasing nutrients like nitrogen, phosphorus, potassium, calcium, magnesium, and microelements availability (Olivares-Campos *et al.*, 2012), provides active substances with phytohormonal effects (Domínguez *et al.*, 2010; Calvo *et al.*, 2014; Aremu *et al.*, 2015). These promote fruit growth (Rodríguez-Dimas *et al.*, 2007; Ayala-Tafoya *et al.*, 2020), and induce increased performance (Reyes-Pérez *et al.*, 2017, Ayala-Tafoya *et al.*, 2020).

Ayala-Tafoya *et al.* (2020) discuss that auxins: 1-naphthaleneacetic acid (0.45%)+1-naphthaleneacetamide (1.2%) increased the fruit set percentage, number of fruits per plant, and yield in zucchini. In this case, naphthaleneacetic acid (ANA), even when not improving the fruit set and number of fruits per plant, did promote zucchini yield by increasing the diameter, length, and weight of their fruits. Montaño and Méndez (2009) and Ariza *et al.* (2015) mention that the naphthaleneacetic acid effect has been detrimental to some crop's yield.

CONCLUSIONS

Applying worm castings increases zucchini yield, fruit set percentage, and number of fruits per plant. Due to the naphthaleneacetic acid effect, the diameter, length, and weight of the fruit increased. The treatments did not influence the stem diameter, leaf greenness, and number of leaves variables; In the Prestige cultivar, a higher leaf area index, the dry weight of leaves and whole plant was obtained; while, with the HMX58 cultivar, greater plant height was achieved.

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Colegio de Postgraduados

Fluctuations and volatility of white eggs consumer prices in the Mexico Valley

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ABSTRACT

Objective: To develop a time series model and analyze the characteristic fluctuations of the average white eggs consumer prices in the Mexico Valley (AWECP), quantifying the seasonal and cyclical fluctuations of said prices.

Design/methodology/approach: A Moving Average Time Series Smoothing method was used to reduce the variation of a data set, and separate cyclical, seasonal, and OLS variations to calculate the trend line.

Results: The Seasonal Component (SC) indicates that in autumn-winter the AWECP is volatile above the annual average by 6.3% while in spring-summer it declines by 9.13% on average. The price volatility is largely explained by external factors and the biological process of poultry animals throughout one year. The cyclical component (CC) was 34 months and occurs irregularly.

Limitations on study/implications: This research focused on finding the SC, T, and CC, but not their respective indices. To make short-term forecasts, it is necessary to calculate the indices and compare them to other time series analysis methodologies.

Findings/conclusions: The analysis of the AWECP in the Mexico Valley indicates the presence of SC and an upward CC trend in the time series. The AWECP volatility has harmful effects on the basic basket of the middle and lower-class population in the Mexico Valley given that this food is the most complete, cheap, and accessible source of animal protein in the Mexican market.

Keywords: Poultry farming, Time series, Moving averages, Price fluctuations, Agricultural economy.

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INTRODUCTION

Chicken eggs are the most complete and cheapest source of protein in the Mexican livestock sector, and due to their competitive price, the most accessible animal protein in local markets (Luis-Rojas *et al.*, 2019). From 1994 to 2021, *per capita* consumption went from 16.7 to 23.7 kg, with an average annual growth rate (AAGR) of 2.08% (UNA, 2021). The *per capita* egg consumption growth in Mexico, despite its volatility, is explained by the competitive price of other protein sources. The Comisión Nacional de Salarios Mínimos (National Commission on Minimum Wages, CONASAMI) set the general minimum wage for 2021 at 141.71 $MXN (CONASAMI, 2021). This amount implies that in Mexico's central economic area, 4.2 kg of eggs or, 2.83, 1.21, and 1.42 kg of chicken, pork, and beef could be purchased. Indicating that egg protein continues to be the cheapest.

The main producing states in Mexico are Jalisco (55.84%), Puebla (12.77%), Sonora (7.88%), the Comarca Lagunera region (Durango and Coahuila) (5.12%), and Yucatán (4.92 %) from the national total (UNA, 2022).

Feeding, packaging, and labor costs are three of the most important inputs in egg production, representing 63.4%, 5.7%, and 4.4% respectively (UNA, 2022).

Tomek and Kaiser (2014) point out that the raw material price behavior results from a complex mix of changes, associated with trend, seasonal, cyclical, and random factors.

According to García-Mata *et al.* (2003), the price volatility and instability that characterize agricultural product markets are due to the derived demand and primary supply being typically inelastic, supply partially depends on random processes (climate, diseases, among others). It is frequently seasonal in nature because producers and dealers must use expected prices for decision-making and expectations introduce systematic changes in prices and quantities.

Separating the components that generate characteristic price fluctuations is important since it permits assessing the nature of the fluctuation, determines whether certain non-random patterns occur or not, and allows isolating and studying each of its components (Martínez-Jiménez and García-Salazar, 2020).

The objective of this research is to develop a moving average time series smoothing model to analyze the characteristic fluctuations of the average price of white eggs to the consumer in the Valley of Mexico (AWECP) and quantify the seasonal, cyclical, and random fluctuations of said prices. The proposed hypothesis is that the seasonal, cyclical, and random components predominantly explain the fluctuation of the AWECP that occurred in the Valley of Mexico recently.

MATERIALS AND METHODS

To evaluate the AWECP behavior and calculate its components, we used a historical series of white eggs' monthly prices to the consumer from the Central de Abastos de la Ciudad de Mexico (Mexico City Supply Central, CEDA-CDMX). This market, considered the world's largest, is a reference to consumer and wholesale prices at a national level in Mexico (SADER, 2023). We also used data provided by the Unión Nacional de Avicultores (National Union of Poultry Farmers, UNA) and the Sistema Nacional de Información de Mercados (National Market Information System, SNIIM) of the Secretaría de Economía (Ministry of Economy, SE). The AWECP series covers 22 years (January 2000 to December 2022), said prices are expressed in nominal Mexican pesos per kilogram ($MXN kg^{-1}).

If $P_t = \left(P_1, P_2, P_3, \ldots, P_n\right)$ is a time series of the AWECP, then the univariate approach of historical series states that a historical series $\left(P_t = f(t)\right)$ of prices is made up of four components, Trend (T), Seasonal Variation (S), Cyclical Fluctuations (C), and Irregular or random Movement (E).

The separation of the four components is considered independent of one another, which implies that their estimation will be successive rather than simultaneous (García-Mata *et al.*, 2003), so each component is multiplicatively related as follows:

$$AWECP_{t\alpha} = T_{t\alpha} * S_{t\alpha} * C_{t\alpha} * E_{t\alpha} \quad (1)$$

Where $AWECP_{t\alpha}$ is the average price of white eggs to the consumer in Mexico, for month t in the year α, expressed in real terms, $T_{t\alpha}$ is the Trend component, $S_{t\alpha}$ is the seasonal component, $C_{t\alpha}$ is the cyclical component and $E_{t\alpha}$ is the irregular or random component.

The Time Series Moving Average Smoothing Method

It has the property of reducing a data set variation and consists of using appropriate order movements to separate cyclical, seasonal, and irregular variations, only leaving the trend movement.

Since the $AWECP_{t\alpha}$ series is expressed in months, and because the twelve-month moving average falls between successive months (June and July) instead of the month's center, the centered twelve-month moving average is calculated, as follows:

$$AWECP_7 = \frac{1}{2}\left(\frac{P_1 + P_2 + P_3 + \ldots + P_{12}}{12} + \frac{P_2 + P_3 + P_4 + \ldots + P_{13}}{12}\right) \quad (2)$$

In this research, a twenty-two-year period is considered $t=1,2,3,\ldots,\ n=22$ with twelve months each year $\alpha=1,2,3,\ldots,\ m=22$.

To estimate price fluctuations caused by white eggs prices' seasonality, it is first necessary to obtain the real egg prices $\left(RAWECP_{t\alpha}\right)$, to eliminate the inflation effect on current prices. This was obtained by dividing the nominal (current) price between the national consumer's price index, based on the 2nd half of July 2018 = 100, provided by Banco de México (Equation 3).

The seasonal index (SI) calculation was estimated with the average movement percentage method, which indicates each month's percentage based on a typical month of each year (annual average value) (García-Mata *et al.*, 2003). To obtain the SI, first, the monthly average price of the observations for each year was calculated (equation 4), the relative price was then calculated by dividing the real price of month t in year a by the average price calculated in equation (5), multiplied by 100 (Martínez-Jiménez and García-Salazar 2020).

The monthly SI (*SIt*) is obtained from the previous data, by dividing the sum of the respective monthly relative price of each year by the total number of years of the analyzed period (22), as indicated in equation (6).

Next, each month's real price $\left(RAWECP_{t\alpha}\right)$ is then divided by its corresponding SI to de-seasonally the price of the white egg $\left(SP_{t\alpha}\right)$ for month tβ in year a, as indicated in equation (7).

$$RAWECP_{t\alpha} = \left(\frac{APWEC_{t\alpha}}{PCNI_{t\alpha}} \right) * 100 \tag{3}$$

$$\overline{AWECP_{t\alpha}} = \frac{\sum_{t=1}^{n} RAPWEC_{t\alpha}}{n} \tag{4}$$

$$PPe_{t\alpha} = \left(\frac{RAPWEC_{t\alpha}}{AWECP_{\alpha}} \right) * 100 \tag{5}$$

$$SI_t = \frac{\sum_{t=1}^{n} PPe_{t\alpha}}{m} \tag{6}$$

$$SP_{t\alpha} = \left(\frac{RAWECP_{t\alpha}}{SI_{t\alpha}} \right) * 100 \tag{7}$$

To calculate the trend component (T), García-Mata (2003) states that it may be obtained through ordinary least squares (OLS), this method assumes that the trend can be graphically represented using a best-fit mathematical line, assuming that the previously de-seasonally adjusted price contains the trend component $\left(T_{t\alpha}\right)$ and the random error $\left(u_{t\alpha}\right)$.

In this research, it was estimated using a polynomial function where the de-seasonally adjusted price $\left(SP_{t\alpha}\right)$ is the dependent variable, and the independent variable is the time (x) equation (8).

$$SP_{t\alpha} = \widehat{T}_{t\alpha} + u_{t\alpha} = \beta_0 + \beta_1 X_{t\alpha} + \beta_2 x_{t\alpha}^2 + \beta_3 x_{t\alpha}^3 + \beta_4 x_{t\alpha}^4 + u_{t\alpha} \tag{8}$$

After estimating equation 8, a new series (CI) is generated, resulting from the quotient of the de-seasonally adjusted price $\left(SP_{t\alpha}\right)$ divided by the $\left(T_{t\alpha}\right)$ trend component.

$$C_{t\alpha} * E_{t\alpha} = \frac{SP_{t\alpha}}{T_{t\alpha}} \tag{9}$$

With the new series data, obtained from equation (9), the cyclical component $\left(C_{t\alpha}\right)$ was in turn obtained using the centered twelve-month moving average, that is:

$$C_{t\alpha} = \left(\frac{(CI)_{t,\alpha} + 2*(CI)_{t,1\alpha} + 2*(CI)_{t,2\alpha} + \ldots + 2*(CI)_{t-1,\alpha} + (CI)_T}{24} \right) \tag{10}$$

Where t is months of the year (t=1, 2, 3,..., T=13), the results of the new smoothed series allow us to assess the cyclical behavior and consequently its duration.

RESULTS AND DISCUSSION

The white egg price volatility to consumers in Mexico

The series indicates a strong white egg price volatility to the consumer in Mexico City Supply Central. This price is the starting point for white egg price at a national level. Registering during July 2000, a minimum of 8.13 pesos per kg price, and a 541% maximum price higher than the minimum price in July 2022.

The high coefficient of variation (42.35 %) denotes high price volatility. This is largely explained by the food inflation environment, given that, until the first half of January 2022, the Mexican annual inflation rate was 7.9%. This, in addition to the avian influenza impact in 47 of the 57 states of the United States of America (USA), which forced the sacrifice of 57.9 million birds, causing smuggling from Mexico to the USA (El País, 2023), and consequently, an excessive price increase in this food for Mexican consumers (Table 1).

Seasonal Component (SC)

The SC shows the percentage increase or decrease that the SC produces in each month of the year. The SI reaches its maximum values from December to January, coinciding with the beginning of winter, respectively. That is, those are the months in which the egg supply in markets decreases, which makes the SC have its greatest price impact.

Throughout the year, white egg prices to consumers had a marked seasonality, a product of climatic variation that exists throughout the year, which impacts the biological process in hens. This results in supply variations, which have an impact on white egg consumer prices in Mexico City.

During autumn-winter, mainly at the beginning of March, the price remains 6.32% above the average, since production is reduced by the season due to the cold weather. In turn, June reports a 9.13% minimum below the average. This is because production increases, due to spring-summer favorable conditions increasing production (Figure 1).

The SC in white egg prices to consumers in the Mexico Valley is present in its characteristic price fluctuations. The price seasonality is explained by the physiological and climatological characteristics of birds, which require heat hours for laying, consequently impacting optimal egg production as well as the market demand uniformity. internally throughout the year. Therefore, it is not possible to influence and mitigate this effect, although there may be other alternatives to reduce the price volatility impact on said product.

Table 1. Statistical indicators of the nominal retail price of white eggs to consumers in the Central de abasto de la Ciudad de México (Mexico City Supply Central, CEDA), January 2000 to December 2022.

	Mean	Median	Mode	Minimum	Maximum	Standard deviation	Coef. of variation
	Mexican pesos per kilogram						
Mexico City	21.08	20.45	30.00	8.13	44.00	8.93	42.35

Source: prepared by authors.

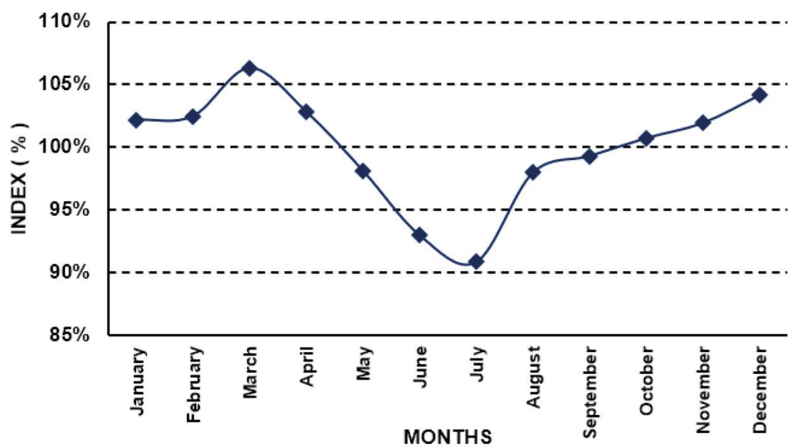

Figure 1. Seasonal index of monthly real price $(P_{t*=E})$ of white eggs to consumers in (%), July 2000 - June 2022.

The seasonal variation of the real consumer price of white egg (RCPWE) oscillates between 20% above and 15% below the average price. However, there are atypical (aberrant) data outside that range, such as those during May 2012, with a 33% drop below the average price, while in April 2020 a 29.6% increase occurred. In the first case, due to supply excess, causing the price decrease, while in the second, due to the beginning of the confinement procedures for the COVID-19 pandemic control in Mexico, causing panic buying, dragging this food price upward (Figure 2).

Trend Component (T)

The AWECP series shows an upward trend, masked by a marked seasonality product of climatic variation throughout the year (García-Mata *et al.*, 2003) (Figure 3).

Parameters $\hat{\beta}_0, \hat{\beta}_1, \hat{\beta}_2, \hat{\beta}_3$, and $\hat{\beta}_4$ were calculated using Ordinary Least Squares, this method provides small error intervals, because the method itself minimizes them. For

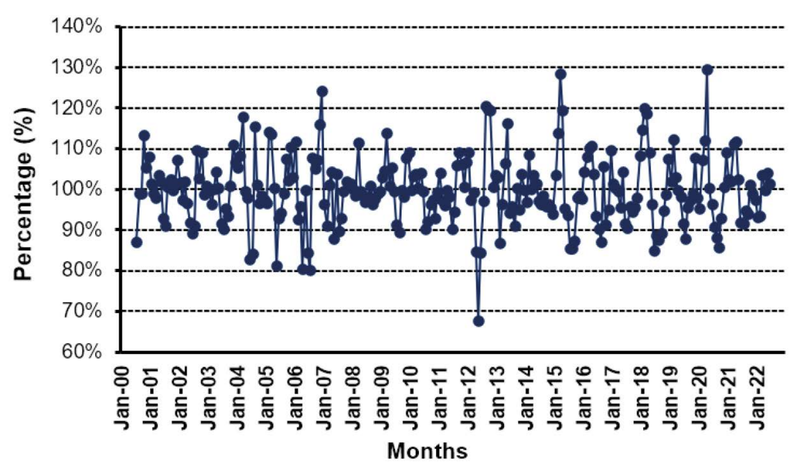

Figure 2. Seasonal variation of real monthly prices $(P_t = S)$ of white eggs to consumer in (%), July 2000 - June 2022.

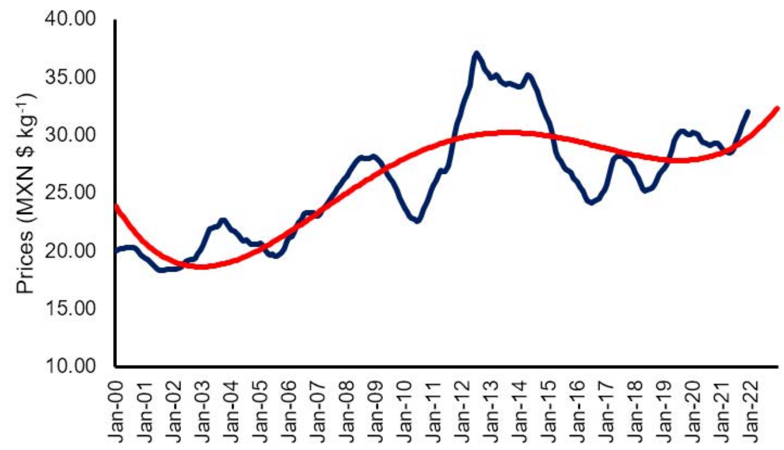

Figure 3. Monthly adjusted prices to white egg consumers trend, 2000 – 2022.

a moderate model to be considered, those parameters whose absolute *t*-statistic is greater than 2 or whose *p*-values are less than 0.05 must be included. Therefore, beta estimators are highly significant.

The coefficient of determination (R^2=0.706) indicates that the regression model explains 70.6% of the Y variable variance (RWECP), so it is assumed that, if more variables are added to the model, this value may increase, decreasing the dependent variable variance. Given that the absolute value of the calculated F (155.76) is greater than the F table value, the null hypothesis is rejected and concluded that the "Y" (RWECP) variable depends on the variable "X" (time), that is to say, it is highly significant (Table 2).

Cyclic Component (CC)

The relevant months that define the RWECP behavior cycles are presented. The CC is also present in white egg prices. For the Mexico City supply central prices, the maximum cycle point occurs during January- June; the minimum is during July-December.

The length of a cycle in price fluctuation goes from a maximum to a minimum, or from a minimum to a maximum. Based on this, from 2000 to 2022, eight complete cycles are observed for prices in the Mexico Valley. The first from May 2001 to January 2002, the second from January 2004 to March 2006; Finally, the eighth cycle from April 2020 to October 2021. On average, the cycle duration is 34 months (2 years 10 months) (Table 3).

Table 2. Trend estimation for the RAPWEC series by ordinary least squares (OLS).

Dependent Variable	Intercept	Independent variables				R^2	Calculated F	Prob>F
Trend (RWECP)	Intercept	X1	X2	X3	X4	0.706	155.76	<0.0001
	24.189	−0.345	0.0065	−3.576E-05	6.152E-08			
Standard error	1.099	0.052	0.0007	3.971E-06	7.117E-09			
t ratio	22.00	−6.64	8.84	−9.01	8.64			

Table 3. Economic cycle duration of white eggs real monthly consumer prices, 2000-2022.

Price maximums and minimums	Average cycle length (months)	Duration of the complete cycle (months)	Duration of the complete cycle (years)
MAX may 2001			
	8		
MIN january 2002		32	2 years 8 months
	24		
MAX january 2004		50	4 years 2 months
	26		
MIN march 2006		40	3 years 4 months
	14		
MAX may 2007		17	1 year 5 months
	3		
MIN august 2007		20	1 year 8 months
	17		
MAX january 2009		40	3 years 4 months
	23		
MIN december 2010		51	4 years 3 months
	28		
MAX april 2013		39	3 years 3 months
	11		
MIN march 2014		15	1 years 3 months
	4		
MAX july 2014		34	2 years 10 months
	30		
MIN january 2017		43	3 years 7 months
	13		
MAX february 2018		23	1 year 11 months
	10		
MIN december 2018		26	2 years 2 months
	16		
MAX april 2020		34	2 years 10 months
	18		
MIN october 2021		18	1 year 6 months
AVERAGE	**17**	**34**	**2 years 10 months**

Regarding the strategies to face the cycles in the characteristic price fluctuations of poultry products in Mexico, the Unión Nacional de Avicultores (National Union of Poultry Farmers, UNA) was created in 1958. This organization is in charge of studying and implementing all the necessary measures to solve the technical, economic, and social problems related to poultry production and distribution in Mexico (UNA, 2022).

The cycles in plate egg prices are closely related to the variation in their input prices. In products such as plate eggs, about 63.4% of production costs are dollarized, which causes the costs of raw materials to increase and, as soon as possible, producers pass food costs to the ultimate consumer (Luis-Rojas *et al.*, 2019).

In the plate egg market, the planning of egg and chicken production through the purchase of grains from national or international producers in a regulated manner through import quotas, as well as vertical integration and technological progress of Mexican poultry farming have been important factors for unit costs to decrease, so supply is displaced faster than demand by population and income.

The genetic improvement of birds has made poultry farming capable of producing a greater product volume with the same amount of input food, and therefore with the same total costs and lower unit costs. The technological change in this activity contributes to the RAWECP having a downward trend in real-term prices; however, the irregular components (diseases, earthquakes, inflation, etc.) alter prices at the producers' level and consequently to the consumer, vulnerating agricultural planning.

CONCLUSIONS

The analysis of consumer prices for white eggs in the Mexico Valley indicates the presence of the SC. The CC upward trend in the time series, according to that proposed by the price components of agricultural products. The AWECP volatility has harmful effects on the basic basket prices for the middle- and lower-class population in the Mexico Valley, since this food is the most complete and cheapest source of protein from the livestock sector in Mexico. Due to the price competition being the most accessible animal protein in the Mexican market, hence a necessity to assess these components to suggest policies that allow avoiding the seasonal and cyclical components of the consumer price of white eggs for dishes in the Mexico Valley.

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Synthesis of silica chitosan oligosaccharides nanoparticles

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ABSTRACT

Objective: To obtain chitosan oligosaccharides (COS) and evaluate COS uses for the obtention of nanosystem based on silica as vehicle and compare the COS-silica nanosystem with the chitosan (Chi) precursor system as Chitosan-silica nanosystems.

Design/methodology/approach: A combination of hydrolysis chemical and mechanical (microwave assisted) were used to obtain COS with the oxidative action of hydrogen peroxide. Sol-Gel adapted method was used to synthetize silica nanoparticles (SiNPs) from sodium metasilicate and the electrostatic interactions between SiNPs and Chi/COS were used to functionalize the SiNPs surface with Chi/COS.

Results: Nanosystem composed from COS and SiNPs were obtained successful as A COS-SiNPs and C COS-SiNPs with particle size of 139.35 nm and 251.8 nm and zeta potential of 30.40 mV and 34.67 mV respectively with antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Limitations on study/implications: Stabilize the systems compound of chitosan-silica nanoparticles due to the molecular weight of chitosan which loss the stabilized the SiNPs suspension and due the incompatibility of both systems pH.

Findings/conclusions: COS and COS-SiNPs stable systems were obtained with an improvement of the antimicrobial activity of the system in contrast of Chi-SiNPs systems.

Keywords: chitosan, silicon, Sol-Gel. Chemical hydrolysis, mechanical hydrolysis, microwave assisted.

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INTRODUCTION

Chitosan is considered as the second most abundant polysaccharide followed by cellulose; chitosan is a biodegradable biopolymer derived from chitin by a deacetylation process. Chitin/Chitosan can be obtained from crustaceous exoskeleton, insects, algae, and from the cell wall of some fungus. Chitin is deacetylated and depolymerized to obtain chitosan in alkaline conditions where acetyl groups are delinked from the saccharide chain, thus reducing the affinity of the polysaccharide to stay attached making chitosan more water soluble and less viscous [1]. Chitin and chitosan are compounded from the same saccharide monomers whit the main difference in the proportion of the units in the polysaccharide structure, chitin is mostly compound with N-acetyl-D-glucosamine (GlcNAc) with a portion of D-glucosamine units (GlcN) and the saccharide units are joined by a β 1-4 glycosidic bond. In contrast, chitosan is mainly compound by GlcN units with a about 10% content of GlcNAc, the ration between GlcN and GlcNAc units defines the deacetylated grade of chitosan [2-4].

Furthermore, chitosan biodegradability other properties have been studied the last decades, as chitosan biocompatibility, low toxicity, antimicrobial effect due their amine groups ($-NH_2$), also chitosan have been studied in agricultural fields due their activity as elicitor in plant defense mechanisms hence chitosan can act as antimicrobial biopolymer coating which activates some defense mechanisms in plants as ethylene and salicylic acid pathways, two of the most important signaling events involving in the resistance of some plant-pathogen interactions as coffea species with *Hemileia vastatrix* (biotrophy pathogen cause coffee leaf rust disease) [5, 6].

The main properties who define the behave and applicability of chitosan are the deacetyl grade, the molecular weight, the availability of amine groups (NH_2) and the zeta potential due the electrostatic charge difference between the biopolymer and the pathogens cell wall [2, 3]. The chitosan can be categorized in function of their molecular weight (Mw) as low molecular weight when Mw<100 kDa, medium molecular weight when Mw<100-1000 kDa and high molecular weight when >1,000 kDa [4].

Besides the widely chitosan applications have inconvenient to scale and improve applications in nanosystems, chitosan solution usually shows high viscosity values and low water solubility in absent of acid environments which reduce their viability to design nanosystems with chitosan, especially if the vehicle particle has more stability in alkaline environments as silica nanoparticles obtained from sodium metasilicate [4, 7].

In contrast chitosan oligosaccharides (COS) have low viscosity values related to their low molecular weight lower than 3.9 kDa and their low polymerization grade, as the COS are obtain from Chitosan by the glycosidic bond hydrolysis, the COS conserve the saccharide units GlcN and GlcNAc which conform chitosan chain but in less length chain and with more functional groups available who are related to COS more water solubility in neutral pH solution with less viscosity, the hydrolysis of glycosidic bond results saccharide fragments of the original chain, at the breaking sites one fragment results in hydroxyl group and the other in aldehyde group [4, 8].

COS can be obtained by chemical, mechanical or enzymatic hydrolysis. Chemical hydrolysis can be divided into acid and oxidative hydrolysis. In acid hydrolysis high concentrations of acids is used where the break of glycosidic bond starts in the protonation of oxygen in glycosidic bonds followed by the group reduction by the addition of water molecule which results in the decomposition of the glycosidic [4, 9, 10]. The oxidative hydrolysis is bases in the uses of hydrogen peroxide to produced hydroxyl radicals due H_2O_2 high instability and attack the glycosidic linkages of chitosan [4, 11].

Mechanical hydrolysis can be combined with acid or oxidative hydrolyses to accelerate the reaction. Mechanical methods can be found microwave and gamma radiation assisted methods where the microwave assisted hydrolysis is less efficient in contrast to gamma radiation but significantly reduces their cost operation, environment impact and scalability. Microwave assisted operates in two ways can generate shear stress and thermal degradation of the polysaccharide [4, 9, 12].

Nanomaterials has gained research attention recently due their wide application in several industries due their behavior and activity change at nanoscale in contrast of their bulk material properties and due it versatility to design more nanomaterials to obtain different morphologies, arrangements or surface modification [13-15], especially in agronomy has ben used to control plant pathogens as insects *e.g.* to control *Tribolium castaneum* with copper nanoparticles (CuNPs) [16-18], and other diseases as Coffee Leaf Disease (CLF) caused by *Hemilea vastatrix* [5], additionally, nanomaterials has been used un food packaging to improve food quality [19], and to improve seed germination [20-22].

SiNPs has special attention due its versatility to obtained in different shape, arrangement and surface chemistry, SiNPs can be obtain from several methods and silicon sources, the mainly used methods are based on Sol-Gel synthesis as the main bottom-up method used due its facilities to control the surface particle chemistry, shape, surface charge and particle size and usually silicon metasilicate or tetraethyl orthosilicate are used as silicon source [7, 13, 19, 23, 24].

MATERIALS AND METHODS.

Silica nanoparticles synthesis

Silica nanoparticles (SiNPs) were synthetized as we report previously by Sol-Gel method [7] sodium metasilicate 0.3M solution distilled water (Metso pentabead 20®, commercial grade) was used as silicon source, the sodium metasilicate solution was filtered with ion-exchange resin to decrease the sodium ions in the solution and reduce the pH from 14 to 10, followed 5 ml of ethanol (Fermont®, analytic grade) was added to catalyzed the condensation of siloxane bonds and leave 15 min in low stirring. The previous solution was homogenized in 2,000 rpm for 30 min with PEGlynated silicon surfactant with methoxy terminal groups (Silwet® L-77, Momentive) to improve micelle formation, finally the obtained suspension was treated in 105 °C reflux for 30 min to improve stability to the suspension.

Silica-chitosan nanoparticles synthesis

Low molecular weight chitosan (Sigma-aldrich®) was dissolved at 3 wt% in previously prepared acetic acid (Fermont®, analytic grade) 1% in distilled water at 70° in vigorous stirring until the chitosan was completely dissolved. To synthetize the silica-chitosan nanoparticles (SiChiNPs) the previously chitosan solution was added into the homogenization step described above with the treated sodium metasilicate and PEGlynated silicon surfactant.

Silica-chitosan oligosaccharides nanoparticles synthesis

To synthetize silica-chitosan oligosaccharides nanoparticles (SiCOSNPs) the previously synthetized SiNPs was dispersed in distilled water at 0.5% with slow stirring, followed obtained COS was added in two different final concentrations 0.5% and 1% and let in agitation for 1 h, the obtained suspensions were labeled as ASiCOSNPs and CSiCOSNPs respectively.

Characterization

The particle size and zeta potential were obtained in particle size analyzer Litesizer 500 (Anton Paar®). The IR spectrum was obtained in ATR-FTIR 4300 (Agilent®), the samples were dried above the ATR to avoid the water molecules interference. The viscosity was obtained at 25 °C by viscosimeter VISCO QC 100 (Anton Paar®). The inhibitory effect of obtained nanoparticles was analyzed by antibiogram method in disposable Petri dishes with previously prepared Mueller-Hinton agar (BD Bioxon®) against *Escherichia coli* ATCC® 25922 and *Staphylococcus aureus* ATCC® 25923.

RESULTS AND DISCUSSION

Viscosity

The viscosity measurements (Table 1) show a significative decrement from the original chitosan solution when the hydrogen peroxide (Table 1. COS H_2O_2) was added from 41.60 P to 3.2 P and more decrement after the microwave treatment (Table 1. COS H_2O_{2-m}) to 32.39 cP. The viscosity decrement can be related to the reduction of molecular weight of the chitosan and the results shows after the microwave treatment confirm the synergic degradation by the combination of chemical and mechanical hydrolysis to obtain chitosan oligosaccharides [8, 10-12].

Fourier-transform infrared spectroscopy (FTIR)

The IR spectrum show similar behave between the dried chitosan spectrum (chi s) and the dried chitosan oligosaccharide spectrum (cos s) with the main difference of the cos s spectrum shows more definition in it peaks spectrum, which according with [25] this can be related to the increase of available functional groups due the glycosidic bond break. Both spectrums start at 650 cm^{-1} regions with stretching of C-O-C and C-O-H bonds at 656.7 cm^{-1} and 652.2 cm^{-1} for chi s and cos s respectively which can be related to the breaking of glycosidic bonds and protonation of the CO^- groups [25].

Both spectrums show well define peaks at 887.1 cm^{-1} (chi s) and 890.8 cm^{-1} (cos s) with similar transmittance which with the peaks at 1012-1016 cm^{-1}, 1052-1060 cm^{-1}, and 1152 cm^{-1} are related with the habitual behave of saccharide structure [26, 27]. The peaks at 1258-1262 cm^{-1} and 1374-1377 cm^{-1} can be related with the presence of amide III associated with GlcNAc units [1, 26-29], the peaks at 1403-1420 cm^{-1} are related to bend vibration of O-H and C-H bonds [1, 26, 27, 30], the presence of peaks around 1531-1539 cm^{-1} are related to vibrations of amide II and C-OH bonds [1, 26, 27], the peaks in 1632-1634 shows the presence of amide I and the peaks around 1700-1702 cm^{-1} are related with carboxyl group vibrations and the peaks at 2866-3069 are related to O-H and C-H bond vibrations [1, 26, 27].

Table 1. Dynamic viscosity related to COS obtention from chitosan.

Sample	Viscosity (Pa)
Chitosan	41.6
COS H_2O_2	3.2
COS H_2O_{2-m}	0.3239

COS H_2O_2 are for COS obtained by oxidative hydrolysis with hydrogen peroxide and COS H_2O_{2-m} for COS obtained by oxidative and mechanical hydrolysis.

The nanoparticles systems synthetized with silica nanoparticles do not show significant differences with the previously discussed spectrums which can be due to a signal overlap between similar bond energy. The siloxane bonds (Si-O-Si) could be overlapped by the glycosidic bond in the saccharide (C-O-C) at 467-586 cm^{-1} respectively and in 1000-1062 cm^{-1} peaks, the peak at 892 cm^{-1} also can be attributed to the bonds Si-C and NH_2, the peaks at 2000-2332 cm^{-1} can be related to S-H or C=O bonds [7, 25, 31].

Dynamic Light Scattering Analysis

Dynamic Light Scattering Analysis shows an increment in size of nanoparticles silica (Figure 2. A) well defined and monodispersed histogram presents a particle size of 6.74 nm with zeta potential (ζ) of −32.80 mV related to deprotonated hydroxyl groups rich in the surface of SiNPs. The first A-Si-COSNPs (Figure 2. B) shows an increment in particle size to 251.8 nm with ζ=30.40 mV which reflects the formation of coat made of COS at the surface of SiNPs. Similar results are shown by C-Si-COSNPs (Figure 2. C) with particle size increment to 139.35 nm and ζ=34.67 mV. By DLS results the nanosystem synthetized with 0.5% of COS (C-Si-COSNPs) can be correlated to more effective and stable nanosystem thus related to the increment of potential zeta in 4.27 mV which reflects more repulsive forces between particles and the smaller particle size (139.35 nm) improve the efficiency of the nanosystem. Additionally, the nanosystem C-Si-COSNPs shows a distribution of size particles more related to monomodal nanoparticles system. The DLS results in Chi-SiNPs systems were discarded due to the trend of the system to precipitate due to molecular weight and incompatible pH regions of chitosan an SiNPs [7].

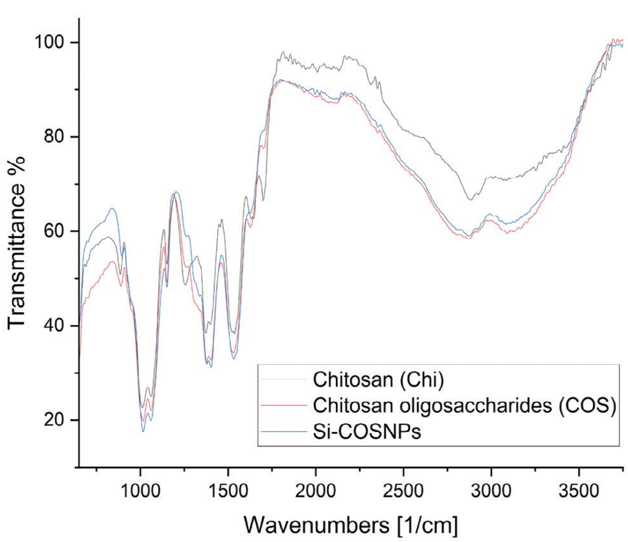

Figure 1. ATR-IR spectrums for Chitosan (Chi), Chitosan oligosaccharides (COS) and Silica-Chitosan Oligosaccharides Nanoparticles (Si-COSNPs).

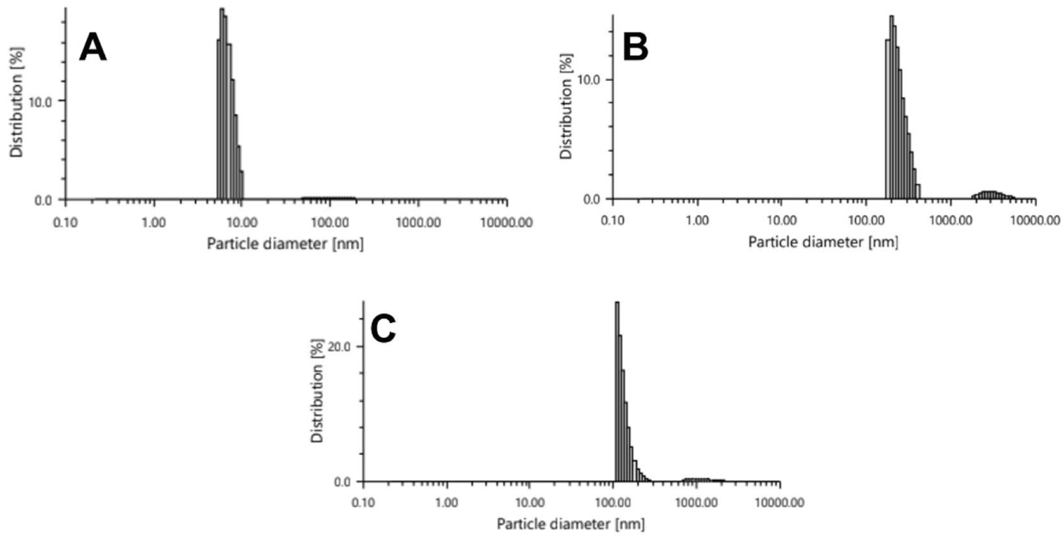

Figure 2. Particle size histogram for SiNPs (A), A-Si-COSNPs (B) and C-Si-COSNPs (C).

Inhibitory efficacy - antibiograms

The obtained chitosan oligosaccharides show inhibitory diameter significant bigger at the 24 and 48 h after the inoculation with *E. coli* and *S. aureus* then the chitosan solution and then silica nanoparticles (Table 2), the silica chitosan oligosaccharide nanosystems (A and C) also show significant inhibitory efficacy in contrast of the silica chitosan nanosystem. The improvement of antimicrobial activity of COS and COS-SiNPS can be related to the increment of amine and amide groups available after hydrolysis of glycosidic bonds, especially due to the increase of primary amino groups [32]. Also, pristine silica nanoparticles show zero-antimicrobial activity as was expected due SiNPs surface negative charge, in contrast, due the positive surface charge shows by COS and COS-SiNPs can be attracted and bonded to bacterial cell wall with negative charge due the carboxylic acid groups and this interaction can makes an impermeable coat of COS or COS-SiNPs blocking the nutrient uptakes and leading to the cell death [32, 33].

Table 2. Antibiogram results of tried nanosystems and precursors against *Escherichia coli* and *Staphylococcus aureus*.

Sample	*Escherichia coli*		*Staphylococcus aureus*	
	24 h (mm)	48 h (mm)	24 h (mm)	48 h (mm)
SiNPs	0	0	0	0
Chitosan	1.9	1.4	1.9	1.4
COS	2.8	2.6	2.8	2.6
SiNPs-COS A	1.9	1.8	1.9	1.8
SiNPs-COS C	1.7	1.6	1.7	1.6
SiNPs-Chi	0	0	0	0

CONCLUSIONS

A stable silica chitosan oligosaccharide nanosystem was successfully obtained with antimicrobial activity against *E. coli* and *S. aureus* by the synthesis of COS from low molecular weight chitosan by chemical and mechanical hydrolysis with hydrogen peroxide and 700W microwave. The obtained nanosystems maintain their main chemical composition characteristic of chitosan with more availability of functional groups due the breaking of glycosidic bond during the COS synthesis. The obtained COS-SiNPs shown a particle size 139.35 nm and ζ=34.67 mV with growth inhibitory diameter of 1.9 mm and 1.8 mm against *E. coli* 24 h and 48 h the inoculation, and 1.9 mm and 1.8 mm against *S. aureus* 24 h and 48 h the inoculation.

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Colegio de Postgraduados

Effect of halophilic bacteria as a sustainable strategy to reduce soil salinity in raspberry (*Rubus idaeus*) cultivation

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ABSTRACT

The raspberry (*Rubus idaeus*) is the main crop of Jocotepec municipality, being dedicated to protected agriculture, chemical fertilization causes soil salinization, which results in the crop's not developing its maximum potential. To evaluate the effect of halophilic bacteria as a sustainable strategy to reduce soil salinity in raspberry cultivation, the present research assessed a production unit located at the Jocotepec Municipality, Jalisco state, Mexico. The evaluated halophilic bacteria treatments were doses of 7 L/ha, 5 L/ha, and 3 L/ha plus an absolute control, on three intervals - of 15 days application in saturated furrow irrigation. The assessed soil variables were pH and EC; in the plants: plant height, number of loaders, stem diameter, and number of boxes produced. The evaluation of the effect of halophilic bacteria on the quantitative variables was carried out with an ANOVA in the SAS® Studio 2023 software, in a completely randomized block design, after complying with normality of residuals and homogeneity of variances assumptions associated with the Tukey means comparison statistical test ($p<0.05$). The results show a direct effect of the halophilic bacteria dosage on the soil EC decrease, from 2.35 ds/m (Control) to 1.81 ds/m (Dose 5 L/ha), values equivalent to light salinity (<2 ds/m). Regarding the agronomic variables, improvements were evident in the evaluated characteristics.

Keywords: Halophilic bacteria, salinity, electrical conductivity

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INTRODUCTION

In Mexico, from a socioeconomic point of view, raspberry cultivation is important because of the employment and foreign exchange it generates, because of its exports as a fresh, frozen, and processed product for consumption. The main producing states are Jalisco, Michoacan, Baja California, Guanajuato, Puebla, Mexico, Colima, Tlaxcala, and

Mexico City with a production of 165,677 t (SIAP, 2022). When this crop grows in salinity conditions, at levels exceeding its tolerance, its plants decrease its growth rate, number of leaves, leaf area, and fruit production (Garriga *et al.*, 2015). In Jocotepec municipality, one dedicated to protected agriculture, the main crop is raspberry, from which chemical fertilization causes soil salinization, consequently affecting this cop developed and maximum potential in the area. When the crop grows in salinity conditions, at levels that exceed its tolerance, the plant decreases its growth rate, the number of leaves, leaf area, and fruit production (Garriga *et al.*, 2015). Aniket and Sengupta (2014) state that agriculture improvement depends on various environmental parameters such as soil temperature, soil moisture, relative humidity, soil pH, light intensity, EC, soil fertilization characteristics, etc. This causes yield degradation. Salt-tolerant microbes introduction is an alternative strategy to crop breeding to increase salt tolerance that improves crop growth (Dodd and Pérez-Alfocea, 2012). Identification and utilization of salinity-tolerant microorganisms could not only improve crop salt tolerance but also reduce pressure on arable land. Among these plant-associated microorganisms, plant growth-promoting rhizobacteria (PGPR) have effectively improved plant stress tolerance (Etesami and Beattie, 2017; Etesami, 2018). Therefore, the objective of this research was to evaluate the effect of halophilic bacteria as a sustainable strategy to reduce the electrical conductivity in saline soil for raspberry (*Rubus idaeus*) cultivation.

MATERIALS AND METHODS

The present investigation was established in a production unit located in the Municipality of Jocotepec, Jal., in which a randomized block design with four treatments and three repetitions was used. The treatments evaluated were based on aerobic halophilic bacterial strains from a consortium formulated in a liquid medium developed under a strict fermentation process of specific microbial strains, with a concentration of heterotrophic bacteria of 2×10^9/ml and Actinomycetes of 2×10^9/ml, with the doses of 7 L/ha, 5 L/ha, 3 L/ha plus an absolute control, three applications were made with intervals of 15 days, at 15, 36 and 57, applying during irrigation, saturating the furrow. The variables evaluated were soil EC and pH; in the plant, plant height, number of loaders, stem diameter, and volume of boxes produced. For treatment, phytotoxicity parameters were taken in the crop, and their agronomic interpretation in the corresponding phenological stage, by the scoring scale proposed by the European Weed Research Society (EWRS). The evaluation of the effect of the treatments on the response of the quantitative variables was carried out through an ANOVA in SAS® Studio 2023 after meeting the assumptions of normality of residuals and homogeneity of variances, associated with the comparison of Tukey means ($p<0.05$) to determine the effect on soil condition, as well as the biostimulant effect on vegetative development.

RESULTS AND DISCUSSION

Forty-two days after application, an analysis of variance and Tukey's comparison test (Table 1) established that all treatments with halophilic bacterial strains had a direct effect on decreasing the soil electrical conductivity ($P<0.05$), which indicates the influence of the treatments on the soil. Five L/ha doses of the evaluated halophilic bacterial strains showed their ability to capture sodium and mobilize it. Their growth is sodium ($Na+$) dependent, and used for various bioenergetic reactions, therefore, constantly mobilized to maintain a (sodium) ions gradient across their cytoplasmic membranes through primary and secondary transport systems (Oren, 2008; Müller & Saum, 2005; Faraj *et al.*, 2016).

The sodium mobilization was evidenced by the electrical conductivity decrease in the evaluated soil, from 2.35 ds/m - corresponding to moderate salinity - to 1.81.0 ds/m - which corresponds to light salinity. Regarding the variables associated with vegetative development in raspberry plants, significant differences are present in all the determined agronomic variables (Table 1), showing the control having lower height, chargers, and stem diameter as well as symptoms of chlorosis and wilting of some plants. Salt-tolerant PGPRs have the potential to stimulate plant growth and productivity by increasing plant nutrient availability, phytohormones, and nitrogen production (Podell *et al.*, 2013). In the variable directly associated with production, there were significant differences in the Tukey tests between the different treatments, reaching the highest number of harvested boxes at a 5 L/ha dose of inoculant halophilic bacteria, with 2953 boxes. While the control reached only 2046.67 boxes. The halophilic bacteria classified within the BPCV directly or indirectly influence through biological nitrogen fixation (BNF), phytohormones synthesis (phytostimulation), increased nutrients availability, P, K, and Zn solubilization, production of siderophores, which reflected as increase crop yields (Mushtaq *et al.*, 2021).

CONCLUSIONS

Based on these results, halophilic bacteria induce a positive response in the variables associated with the soil, such as pH and EC. The evaluated 5 L/ha dose statistically shows to be the best treatment, since it induced better plant height, number of loaders, stem diameter, and volume of boxes produced responses. The halophilic bacteria at the doses evaluated had no phytotoxic effects in raspberry plants, classified as 1 (No effect on the crop) on the EWRS scale. Inoculation of halophilic strains promotes plant growth and

Table 1. ANOVA and Duncan Grouping on each variable 42 days after application.

TRAT	VARIABLES											
	pH*		EC*		Ph*		NUM CAR*		DIASTEM*		NB*	
	CV=0.3932		CV=0.4204		CV=1.009		CV=0.1388		CV=2.08		CV=0.1067	
	Average	cluster	Average	cluster	Average	cluster	Average	cluster	Average	cluster	Average	cluster
0 L/ha	7.030	A	2.3500	A	1.5333	D	11.1100	D	0.6267	D	2046.67	D
3 L/ha	6.550	B	2.1500	B	1.5900	C	11.8200	C	0.7000	C	2451.00	C
5 L/ha	5.667	D	1.8100	D	1.7700	A	15.2000	A	0.9600	A	2953.00	A
7 L/ha	5.840	C	1.9300	C	1.7300	B	15.1267	B	0.9767	B	2913.14	B

Source: SAS® Studio 2023. CV: coefficient of variation. pH.- soil pH value. EC.- soil Electrical conductivity. Ph-. Plant height. NUM CAR.- Number of chargers. DIASTEM.- stem diameter. NB.- Number of harvested boxes.

reduces salt stress in raspberry cultivation. This may be because they can reduce soil EC and, therefore, the salinity concentration. These results show the possibility of recovering soils with salinity and/or sodicity problems through biological processes based on halophilic microorganisms.

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Colegio de Postgraduados

Heterosis in the germination process and seed characteristics of the maize hybrid (*Zea mays* L.) HAZUL 10E

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ABSTRACT
Objective: To quantify the heterosis in physical and physiological characteristics in seeds of simple and trilinear crosses of the HAZUL 10E corn hybrid.
Methodology: Seeds of the genotypes conforming to the HAZUL 10E corn hybrid (endogamic lines and simple and trilinear crosses) were used. The experimental design was completely randomized with three repetitions. Physical (weight, width, thickness, length, volume, density, and the width/length and thickness/length ratios) and physiological (normal and abnormal seedlings, death seeds, and lengths and dry matter of plumule, radicle, and total) characteristics of the seeds were evaluated. Heterosis and heterobeltiosis were determined, and the differences between both crosses were statistically tested with Student's t-test.
Results: Heterosis and heterobeltiosis were higher in the single cross than in the trilinear one. In the single cross, the highest values of both heterosis corresponded to the plumule, radicle, and total dry matter; followed by weight and volume seed. In the trilinear, the highest values corresponded to normal seedlings, radicle length, and biomass total. The variables corresponding to shape seed, normal seedlings formation, and radicle elongation, responded better to hybridization in the trilinear cross.
Study limitations: None presented.
Conclusions: In the single cross, heterosis increased the seed size and accumulated biomass in seedlings; while in the trilinear cross, it affected the shape of the seeds and the produced seedlings.

Keywords: Hybrid vigor; pigmented corn; seed germination; seed quality.

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INTRODUCTION

Corn (*Zea mays* L.) is the most important crop in the world by planted area and production volume [1]. In 2021, 7.4 million hectares were planted in Mexico and 27.5 million tons of grain were harvested [2]. Blue corn is relevant for its wide use in traditional

Mexican cuisine (tortillas, tlacoyos, sopes, etc.) [3], due to its exotic color and its attributes of flavor, texture, and nutraceutical benefits [4].

In Mexico's central states (Estado de Mexico, Puebla, Tlaxcala, and Mexico City) 150 thousand hectares of blue corn are planted with native varieties, traditional technology, and rainfed conditions, and 300 thousand tons of grain are harvested, which do not satisfy the demand for this food.

The current grain yield of blue corn is 1.9 t ha^{-1} [2]. It is feasible to significantly increase it through the utilization of hybrids [5] developed on the genetic variability available in the region of interest [6].

At the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (National Institute of Agricultural and Livestock Forestry Research, INIFAP), blue corn experimental hybrids were developed for high valleys (2400-2800 meters above sea level), with yields of 6.4 to 8.2 t ha^{-1}, from which HAZUL10 stood out for producing 8.2 t ha^{-1}, on average, in high, medium and low productivity environments [7].

Hybridization was described at the beginning of the 20th century [8]. Since then, it has successfully improved corn and is currently still applied for obtaining hybrids that help to satisfy the global demand for this grain [9]. With hybridization, the F1 expresses larger morphological dimensions and better grain yield than unrelated endogamic parents [10], to a magnitude dependent on its genetic background and its production environment. The above responses are caused by heterosis, a complex, and poorly understood phenomenon, but widely used in the corn and other basic crops improvement.

The objective of the present research was to quantify heterosis and heterobeltiosis in the physical and germinative characteristics of seeds of simple and trilinear crosses of the HAZUL 10E corn hybrid.

MATERIALS AND METHODS

Genetic material

Seeds from the parental genotypes of the experimental blue corn hybrid HAZUL 10E were used: L11 (BXCC-54-11-1-1-1), L12 (BXCC-5-9-6), L10 (NXOAX-19-5-1-1-2), Simple Cross A (BXCC-54-11-1-1-1) × (BXCC-5-9-6) and the Trilinear Cross B [(BXCC-54-11-1-1-1) × (BXCC-5-9-6)] × (NXO-AX-19-5-1-1-2). In this document, the data of the simple cross (treatment A) and the trilinear cross (treatment B) were presented. The hybrid was developed in the Maize Program of the Valle de México Experimental Field from the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (CEVAMEX, INIFAP).

Physical characteristics

Seed weight (SW). 100 seeds (mg) were weighed on an analytical balance (AE Adam P W 184, precision 0.1 mg). The width (SWID), thickness (ST), and length (SL) of the seeds were measured (mm) with a digital vernier (Mitutoyo CD-6 CSX). The volume (SV, mm^3), relative density (SRD, g/cm^3), and the SWID/SL and ST/SL ratios of the seeds were calculated according to the corn varietal descriptors [11-13].

Germinative characteristics

Normal germination

The standard germination test was used [14]. The seeds were placed in paper towels saturated with humidity in a germination chamber at 25 °C and 100% r. h. After seven days of incubation, the seedlings with complete morphology and free of pathogens (NS), abnormal seedlings (AS), and dead seeds, *i.e.*, lacking metabolic signs, (DS) were counted (%). NS was considered equivalent to seed germination.

Seedlings development

In normal seedlings, the length of plumule (PL), radicle (RL), and total length (TL) were measured (mm); these structures were then, dried in an oven (80 °C, 4 d) and their dry weight (PDW, RDW, and TDW, respectively) was determined (mg).

Heterosis estimation

Heterosis (%) was calculated in relation to the parent's average of the cross

$$H = \left[\left(F1 - AP \right) / AP \right] * 100$$

and also to the best parent (heterobeltiosis)

$$HB = \left[\left(F1 - MP \right) / BP \right] * 100;$$

where $F1$=Value of the cross, AP=Average parents of the cross, and BP=Best parent of the cross [15].

Statistical analysis

Student's paired data test was used to test the significance ($P \leq 0.05$ and $P \leq 0.01$) of the differences in H and HB within each cross, as well as that of the differences in H or HB between the mentioned crosses. The value of this difference was multiplied by (−1) to indicate the variation in the variables [16].

The statistical processing of the results was done with the SAS statistical software (ver. 9.2) [17].

RESULTS AND DISCUSSION

In response to the hybridization of the crossings involved in the formation of the trilinear corn hybrid HAZUL 10E, heterosis (H) exceeded heterobeltiosis (HB) in all measurements, confirmed by the negative values of the difference between both (Table 1). The above is explained because the H was calculated in relation to the average number of parents of the cross and the HB regarding the best parent, *i.e.*, it was more rigorous [15].

Both types of heterosis were higher in cross A than in B, because the genetic recombination occurred between two inbred lines in the first case, and between a simple cross and an endogamic line, in the second. This situation coincides with the argument

Table 1. Values (%) of heterosis (H) and heterobeltiosis (HB) of the simple (A) and trilinear (B) crosses of the experimental blue corn hybrid H10 E (UPIBI, IPN. Mexico City, Mexico, 2020).

Variable	Genotypes							
	A				B			
	H	HB	H−HB	t	H	HB	H−HB	t
SW	63.93	45.07	−18.86	**	13.61	−3.21	−16.82	**
SV	35.01	22.85	−12.16	**	22.44	14.75	−7.69	*
SL	17.31	11.3	−6.02	*	4.79	2.20	−2.59	**
SWID	15.37	13.34	−2.03	ns	7.83	5.86	−1.97	ns
ST	−0.36	−6.07	−5.71	**	9.71	5.35	−4.36	*
SRD	12.95	0.42	−12.53	**	0.05	−10.72	−10.77	**
SWID/SL	−2.06	−8.63	−6.57	**	3.80	3.80	0	ns
ST/SL	−15.07	−18.19	−3.12	**	5.63	5.93	0.3	ns
NS	8.93	−4.58	−13.51	ns	62.61	35.62	−26.99	ns
AS	25	−25	−50	ns	−50.00	−50.00	0	ns
SD	0	−29.58	−29.58	ns	−55.71	−62.50	−6.79	*
PL	18.38	9.02	−9.36	ns	−4.21	−13.79	−9.58	*
RL	8.08	3.28	−4.80	ns	45.52	34.62	−10.9	*
TL	11.38	5.78	−5.60	ns	25.55	19.46	−6.09	ns
PDW	92.16	61.04	−31.12	*	−0.03	−15.45	−15.42	*
RDW	84.79	52	−32.79	*	78.86	38.52	−40.34	*
TDW	87.96	58.18	−29.78	ns	41.68	14.04	−27.64	*

*=Significant (P≤0.05), **=Highly significant (P≤0.01), ns=Not significant. SW=Seed weight, VS=Seed volume, LS=Seed length, WS=Seed width, TS=Seed thickness, RDS=Relative seed diameter, PN=Normal seedlings, PA=Abnormal seedlings, SI=Seeds inert, LP=Plumule length, LR=Radicle length, TL=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, WS/LS=Seed width/Seed length, TS/LS=Seed thick−ness/Seed length.

that the heterotic response depends on the genetic background of the genotypes involved in the crossing [18].

In genotype A, the highest magnitudes of H and HB were noted in the characters corresponding to the dry matter production of the seedlings (PDW, RDW, and TDW), followed by weight (WS) and volume seed (SV); while, for B, the highest values corresponded to germination (NS), biomass (RDW and TDW) and radicle elongation (LR). In these variables, the effects of the metabolic process called heterosis or hybrid vigor were expressed, through which the offspring, in this case, the crosses, assume larger dimensions compared to the parents in certain variables [19, 20], in which the phenotypic ones predominate; However, in the present study heterosis also occurred in physiological ones, as was the case of NS, RDW, TDW, and RL.

Thus, the response to heterosis varied depending on the cross and the measured trait, although total and radicle biomass showed a high response in both crosses. The H and HB values of the NS in genotype B also stood out, this evidenced the favorable genetic recombination of the L10 line, which served as a pollinator for the L11×L12 simple cross, for seed germination, which combined with the heterosis manifested in RL, RDW, and TDW, conferred favorable characteristics for the establishment of the assessed hybrid in the crop field. This result can be attributed to L10 coming from the Central Valleys of Oaxaca state, Mexico, a different environment from that in the Valley of Mexico, in which L11 and L12 were collected; so the lines belong to dissymbol heterotic groups, whose genetic recombination had a positive effect on the physiological quality of HAZUL 10E [18].

In A, the differences between H and HB (Table 1) were significant in 53% of the variables, while in B in 64%; Both crosses concur in that these significances were shown by seed size and dry matter production (SW, SV, SL, ST, SRD, PDW and RDW); this data showed the wide heterogeneity of the L11 and L12 parental lines regard the aforementioned variables, which was balanced considering the average of parents (H) but not by including only the best of them (HB), therefore, the differences were significant.

In the same sense, comparing H or HB between the crosses (Table 2) confirmed that both occurred with a greater magnitude in A than in B. In A, there were significant

Table 2. Differences between heterosis and heterobeltiosis of the simple (A) and trilinear (B) crosses of the experimental blue corn hybrid H10 E (UPIBI, IPN. Mexico City, Mexico, 2020).

Variable	Genotypes							
	A	B	(H−H)	t	A	B	(HB−HB)	t
	H	H			HB	HB		
SW	63.93	13.61	−50.32	**	45.06	−3.21	−48.27	**
SV	35.01	22.44	−12.57	ns	22.85	14.75	−8.10	ns
SL	17.31	4.79	−12.52	**	11.30	2.20	−9.10	**
SWID	15.37	7.83	−7.54	ns	13.34	5.86	−7.48	ns
ST	−0.36	9.71	10.07	ns	−6.07	5.36	11.43	ns
SRD	12.95	0.05	−12.9	ns	0.42	−10.72	−11.14	ns
SWID/SL	−2.06	3.80	5.86	ns	−8.63	3.80	12.44	*
ST/SL	−15.07	5.63	20.7	*	−18.19	5.93	24.12	*
NS	8.93	62.62	53.69	*	−4.58	35.62	40.21	ns
AS	25.00	−50.00	−75	ns	−25.00	−50.00	−25.00	ns
SD	0.00	−55.71	−55.71	*	−29.58	−62.50	−32.92	**
PL	18.38	−4.21	−22.59	ns	9.02	−13.79	−22.81	ns
RL	8.08	45.51	37.43	**	3.27	34.62	31.35	*
TL	11.37	25.56	14.19	*	5.78	19.46	13.68	ns
PDW	92.16	−0.03	−92.19	ns	61.04	−15.45	−76.48	ns
RDW	84.79	78.85	−5.94	ns	52.00	38.52	−13.48	ns
TDW	87.96	41.68	−46.28	ns	58.18	14.04	−44.14	ns

*=Significant ($P\leq0.05$), **=Highly significant ($P\leq0.01$), ns=Not significant. SW=Seed weight, VS=Seed volume, LS=Seed length, WS=Seed width, TS=Seed thickness, RDS=Relative seed diameter, PN=Normal seedlings, PA=Abnormal seedlings, SI=Seeds inert, LP=Plumule length, LR=Radicle length, LT=Total length, PDW=Plumule dry weight, RDW=Radicle dry weight, TDW=Total dry weight, WS/LS=Seed width/Seed length, TS/LS=Seed thick−ness/Seed length.

differences in the variables SW, SL, ST/SL, NS, SD, RL, and TL; while, in HB the significances were for SW, SL, SWID/SL, ST/SL, DS, and LR, *i. e.*, both ways of quantifying heterosis coincided in the variables (except NS, SWID/SL, and TL) that revealed the disparity of the heterotic response caused by the already described dissimilar genetic constitution of A and B.

The analysis of the significances (Table 2) also showed that the ST/SL, NS, RL, and TL variables, in A and SWID/SL, ST/SL, and RL, in B; reached positive and significant values, in contrast to the rest of the variables. So these variables better responded to hybridization in the trilinear cross (B), both in H and HB, which had a positive impact on the shape (SWID/SL and ST/SL) and seed germination (NS), as well as in the length growth of the radicle of the produced seedlings.

CONCLUSIONS

In the simple cross, heterosis increased seed dimensions and accumulated biomass in seedlings; while in the trilinear cross, it affected the shape of the seed and the normal seedlings produced.

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Colegio de Postgraduados

Organic extracts for fall armyworm (*Spodoptera frugiperda*) control in native corn (*Zea mays* L.)

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ABSTRACT
Objective: Determine the incidence of fall armyworm in corn plots and evaluate the insecticidal effect of garlic extract and rue essential oil and understand their phytochemistry.
Design/methodology/approach: The fall armyworm incidence was determined through the "five of golds" systematic sampling, composed of 20 plants per sampling point (100 plants in each plot). This assessment was conducted in 15 plots of native corn in the municipality of Cherán, Michoacán. Control bioassays were also tested, in the laboratory and field; where rue essential oil, garlic extract, cypermethrin, and an absolute control (water) were evaluated.
Results: The highest fall armyworm incidence (18%) occurred in a corn plot at Andasticua. In the laboratory, cypermethrin induced the highest fall armyworm mortality (100%), followed by rue essential oil (83%). In the field, both the insecticide and the rue essential oil reduced lepidopteran incidence. The main component of the essential *R. graveolens* oil is the semivolatyl cinnamoyl chromen (IUPAC name: 2-(3-phenylprop-2-enoyl) chromen-4-one).
Limitations of the study/implications: The present research has no major limitations.
Findings/conclusions: Both products, cypermethrin, and rue essential oil, effectively control fall armyworms in the laboratory and the field environments.

Keywords: Botanical bioinsecticides, active substance, rue essential oil.

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INTRODUCTION

The *Spodoptera* genus is present in all world agricultural regions (EPPO, 2020). Currently, the fall armyworm (*Spodoptera frugiperda*) is the pest with the greatest economic impact on corn cultivation; damaging the plant buds, the stems, when it behaves like a cutworm and can cause the loss of up to 50% of production in Mexico (Blanco *et al.*, 2014). This pest is mainly controlled with chemical insecticide methods, which cause considerable environmental damage, due to irrational utilization (Cano *et al.*, 2004). The indigenous farmers from the Cherán region, Michoacán state, Mexico, point out the need to implement more reasonable control strategies to improve the agroecological environment; on this, Alieri and Toledo (2010) discuss that the basis for achieving this is to start from experimentation processes and rescuing traditional knowledge. That is why this experiment was planned with a more environmentally friendly alternative. In this sense, two natural extracts; one from rue (*Ruta graveolens*), and the other from garlic (*Allium sativum*) were applied to counteract the effects caused by the fall armyworm. Rue extract comes from a perennial herbaceous plant, 50-90 cm high, with round stems and bluish-green foliage. It is frequently used for its medicinal properties and utilized for the control and management of the native corn crops of fall armyworm by the Indigenous community of Cherán, Michoacán state. Due to the above, the objectives were to determine the fall armyworm incidence in native corn plots evaluate the insecticidal effect of garlic extract and rue essential oil, and to know their phytochemistry.

MATERIALS AND METHODS

Study area

The Cherán community is located in the Purépecha Plateau region, 19° 38' - 19° 51' north latitude 101° 52' - 102° 08' west longitude, at an altitude ranging between 2,200 and 3,200 meters above sea level. It is limited to the northeast by Zacapu, to the southeast by Nahuatzen, to the southwest by Paracho, and to the northwest by Chilchota (Prontuario, 2009).

Fall armyworm collecting

Fall armyworms were directly collected, in corn plots, to carry out an *in vitro* bioassay at that moment, Instar L4 and L5 larvae with similar size, thickness, and colour characteristics were collected. Analytical keys were used for the insect identification (Bautista, 2006; Ruiz *et al.*, 2013).

Rue essential oil and garlic extraction

The oil extraction was conducted following the technique by Díaz (2017) with some modifications. First, the rue plants were washed with tap water and dehydrated for 25 days, until completely dry. Rue oil was obtained using the leaves and stems of the plants, by steam extraction using a Clevenger type device. The applied garlic extract was commercial (Garlic extract 87%. SA).

Fall armyworm incidence

The lepidopteran incidence was determined in 15 corn plots GPS located (Table 1). A systematic sampling was carried out, five of golds or X sampling, composed of twenty plants per point, 100 plants in total in each plot. The sampling consisted of selecting r groups of sampling units in each corner of the field and one in the center of it (Domínguez, 1992).

Fall armyworm *in vitro* bioassay

The bioassay was developed at the Phytopathology laboratory of the CIIDIR MICHOACÁN. It consisted of 3 treatments: 1. Rue essential oil (5 mL L^{-1} of water), 2.

Table 1. Location of native corn plots, sampled in Cherán, Michoacán, to determine fall armyworm incidence.

Site No.	Zone	Coordinates		Masl	Place
		Q	UTM		
1	13	0814526	2177962	2,172	Camino viejo de Paracho (El plan)
2	14	0186378	2178575	2,175	Camino viejo de Paracho (El plan)
3	14	0186052	2179000	2,173	Camino viejo de Paracho (El plan)
4	14	0186051	2180246	2,186	Camino viejo de Paracho (El plan)
5	13	0814510	2180285	2,185	Andasticua
6	14	0185638	2180167	2,180	Andasticua
7	14	0187278	2180149	2,197	Andasticua
8	14	0190957	2177243	2,366	Saricho
9	14	0192205	2177000	2,383	La Barricada
10	14	0193325	2180551	2,469	El Borrego
11	14	0185648	2178018	2,173	Camino de Paracho
12	14	0186404	2178566	2,174	Juanyan
13	14	0186019	2178999	2,172	Juanyan
14	13	0814520	2180280	2,189	Camino viejo Cheranastico
15	14	0187281	2180144	2,203	Camino Piedra parada

UTM=Universal Transverse Mercator; Masl=Meters above sea level.

20% CE Cypermethrin insecticide (1 mL L^{-1} of water), and 3. Absolute control (distilled water). Fifty-four fourth and fifth instar fall armyworm larvae were used. The design was completely randomized, with three treatments and three repetitions. Six larvae were considered per repetition, placing three per Petri dish. Subsequently, each of the treatments was sprayed on the larvae, with atomizers. Mortality was evaluated after 6, 12, 18, 24, and 30 h, and the dead larvae percentage was calculated using Abbott's formula (1925):

$$\%\ Mortality = 100\left(\%\ treated\ \ deaths - \%\ control\ \ deaths\right)/100 - \%\ control\ \ deaths$$

Fall armyworm field bioassay

The experiment took place in a native corn plot, in Andasticua, Cherán community, Michoacán. Four treatments were assessed: 1. Insecticide Cypermethrin 20% CE (1 mL L^{-1} of water), 2. Rue essential oil (10 mL L^{-1} of water), 3. Garlic extract (5 mL L^{-1} water) and 4. Control (distilled water). In this experiment, a randomized block experimental design was used, with 4 treatments and 4 repetitions. The experimental unit consisted of four 5 m long furrows. A surfactant dispersant was added in all treatments. Two applications were conducted in a 15-day interval.

The effectiveness degree of the treatments was determined following the Henderson and Tilton (1955) equation cited by Figueroa *et al.* (2019).

$$\%\,efficacy = 1 - \left[\frac{N_t \times M_o}{N_o \times M_t}\right] \times 100$$

Where: N_t=Larvae population in the control group before treatment; N_0=Larvae population in the control group after treatment; M_t=Larvae population in the experimental group before treatment; M_0=Larvae population in the experimental group after treatment.

Rue essential oil phytochemistry

A secondary metabolites identification of the rue essential oil was carried out via mass spectrometry in an ultra-high-resolution liquid chromatograph with electrospray ionization (UHPLC-ESI) of the Centro de Nanociencias y Micro y Nanotecnologías of the IPN.

Statistical analysis

The variables under study were subjected to an analysis of variance and the Tukey statistical test ($p=0.05\%$), to determine the statistical significance between treatments, using the Statistical Analysis System (SAS) software.

RESULTS AND DISCUSSION

Fall armyworm incidence

Fall armyworm was observed in all sampled plots. The highest incidence, 18%, occurred in a plot located at Andasticua (site 7), the average incidence was 8% (Figure 1). This relatively low average incidence is due to some plots located in high areas, at altitudes between 2,203 and 2,469 meters above sea level, where temperatures are lower than those recorded in the lower areas. Research by Valdez-Torres *et al.* (2012) on corn cultivation determined that the average minimum threshold temperature for fall armyworms development is 8.7 °C, and their average optimal development temperature is 25 °C. No larval growth is considered possible when night temperatures are lower than 4.4 °C (Santana *et al.*, 2016) and the percentage of larval survival considerably decreases at lower than 9.8 °C temperatures (Yáñez *et al.*, 2019); which explains the previously stated.

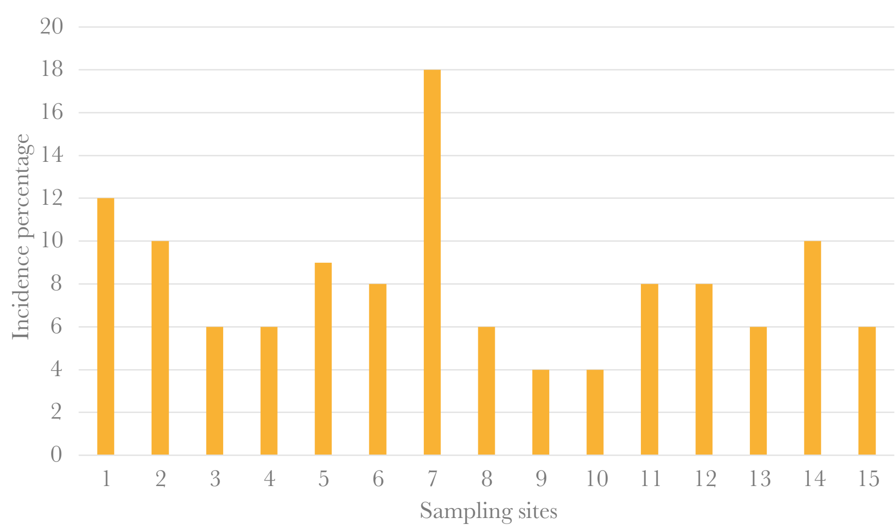

Figure 1. Fall armyworm incidence in 15 native corn plots at Cherán municipality, Michoacán state.

***In vitro* fall armyworm mortality percentage**

The chemical Cypermethrin induced the highest fall armyworm mortality, with significant statistical differences ($p \leq 0.05$). Fifty percent of the larvae treated with this insecticide died within 6 hours after its application. At 12 hours, 100% of the larvae were dead. It is reported that other chemicals, such as Spinetoram, are also capable of inducing 100% fall armyworm mortality in instars 3 and 4 (Miranda, 2016). If rue essential oil is compared with chemical treatments regarding their produced mortality, the latter may be better. However, rue essential oil can be a good prospect for fall armyworm control, because of its fewer environmental effects; contrary to insecticides that constitute a risk factor for terrestrial and marine ecosystems (García-Gutiérrez and Rodríguez-Meza, 2012). This may be because only 10% of the field-applied pesticide reaches the harmful organisms they are intended for and a proportion of them is deposited in the soil, water, and sediments (Ortiz *et al*., 2011). In some countries, for example, Cuba, there are norms and regulations to reduce indiscriminate chemical-origin pesticide use, and on the contrary, implement biological control alternatives (Del Puerto *et al*., 2014).

It is noteworthy that rue essential oil had anti-feeding effects for the fall armyworm, larvae treated with it stopped feeding from the first hours after spraying. Together, stopping feeding and the insecticidal effect that the essential oil could exert caused a mortality rate of 83% (Figure 2). Although the biological effectiveness of any plant extract is not 100% as in this case, these results are satisfactory. Research by Lizarazo *et al*. (2008), of fall armyworm with second instar larvae, concluded that mullein extract (Polygonum hydropiperoides) achieved 100% mortality 12 days after the larvae were sprayed. Likewise, these authors indicated that said extract had an antifeedant effect, given the less than 4% reduction in corn foliage consumption; similar results to those obtained with Chlorpyriphos insecticide. Another research demonstrated that *Solanum elaeagnifolium* aqueous extract had a mortality effect of 47.5% in a 24 to 72-hour period, in second-instar larvae exposed to that extract (Guevara, 2021). Also, the aqueous extract of *Melia azedarach* L. at a 350 g/L concentration

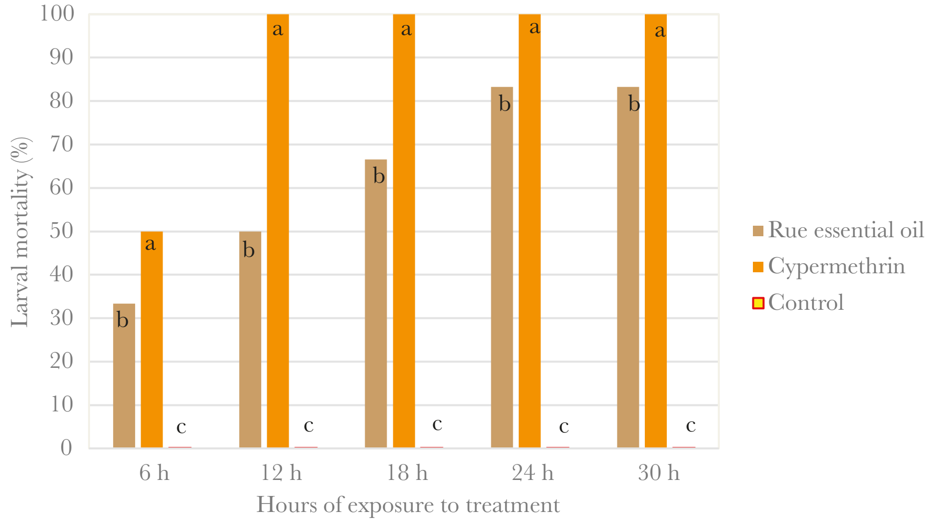

Figure 2. *Spodoptera frugiperda* larvae mortality due to treatment effects.

reported 100% mortality on the sixth day of their bioassays, as well as nutritional indices also significantly decreasing compared to their control (Mimbela, 2013).

Field fall armyworm control

The most successful fall armyworm control in the field was the Cypermethrin insecticide since it had the largest number of larvae deaths. However, it was statistically similar ($p \leq 0.05$) from the rue essential oil treatment, and this in turn with garlic extract treatment (Table 3). Other research concurs with these results and indicates that garlic extract has a biological activity against fall armyworms (Figueroa *et al.*, 2019). Likewise, these researchers point out that extracts from *Azadirachta indica*, *Piper nigrum*, *Petiveria alliacea*, *Nicotiana tabacum*, and *Lippia alba* were also effective in this pest control.

Table 2. Effectiveness degree of field treatments for fall armyworm control in native corn.

Treatments	Number of larvae (1st. Application)			Number of larvae (2nd. Application)		
	Before	After	Effectiveness (%)	Before	After	Effectivenes (%)
Cypermethrin	70	14 c	83	24	2 c	93
Rue essential oil	75	29 bc	67	41	8 bc	84
Garlic extract	71	38 b	54	50	12 b	80
Control	74	86 a	-	101	122 a	-

Different letters in the columns indicate significant differences (Tukey, $p \leq 0.05$).

It is also important to note that the fall armyworm incidence in the experimental field was not high (<15%); which may relate to the region's cooler temperature. Under optimal larval development conditions, life cycles are shorter; therefore, there may be more generations and greater dispersal in each area (Ramírez-Cabral *et al.*, 2020).

Essential oil phytochemistry

The analysis of *R. graveolens* essential oil on its secondary metabolites indicates that the main and majority component is the semivolatile cinnamoyl chromone (IUPAC name: 2-(3-phenylprop-2-enoyl)chromen-4-one) with a mass-charge ratio of 277.0831 m/z.

CONCLUSIONS

The highest fall armyworm incidence, 18%, occurred in a plot located at Andasticua. Cypermethrin, by contact, caused the 100% death of fall armyworms in 12 hours, followed by rue essential oil which reached 83% *in vitro* mortality. Both products, the insecticide, and the rue essential oil, were most effective in controlling fall armyworms in corn in the field. However, essential oils can contaminate the environment to a lesser degree, compared to the insecticide. The main *R. graveolens* essential oil component is the semivolatyl cinnamoyl chromone (IUPAC name: 2-(3-phenylprop-2-enoyl)chromen-4-one).

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Colegio de Postgraduados

Nitrogen fertilization in wheat, in clay soils at the Mexicali valley, Baja California, Mexico

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ABSTRACT
The national and international markets' demand for wheat commercialization is conditioned by quality standards, among which the protein content and percentage of vitreous grain, without white belly, stand out. In them, nitrogen plays an important role in the yield and quality of the wheat grain. For these reasons, the objective of this research was to determine the effect that nitrogen has on yield, grain protein, and the vitreous grain percentage. A field experiment, planted on December 16, 2009, was conducted at the Institute of Agricultural Sciences of the Universidad Autónoma de Baja California. The experimental design was of complete randomized blocks with four repetitions. The assessed treatments were 0, 105, 210, 315, and 340 kg of N ha^{-1} (N_0, N_{105}, N_{210}, N_{315} and N_{340}, respectively). The sown seed was Aconchi F-76 variety crystal wheat. The evaluated variables were grain yield, protein content, and vitreous grain quantity (without white belly). The results indicated that the 210, 315, and 340 kg of N ha^{-1} treatments affected the yield, protein content, and white belly decrease in the grain. Grain quality is therefore improved with these nitrogen doses, in relation to the quality of the harvested grain in the plots with 0 kg ha^{-1} and those cultivated with 105 kg ha^{-1}.

Keywords: nitrogen fertilization, white belly, vitreous grain.

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INTRODUCTION

Worldwide, wheat (*Tritticum vulgare*) is the most utilized cereal in human nutrition. The wheat importance in the human diet mainly lies in its high energy value, plus containing more protein than corn and rice (Peña *et al.*, 2007). According to Evans (1993), worldwide, digestible energy (83.7%) and protein (65%) for human diets come directly from crops, out of which, cereals contribute more than half of the energy diet. After corn and beans, wheat is the third source of nutrients in the Mexican diet, mainly in low-economic urban and rural populations (Peña *et al.*, 2007).

In Mexico, wheat is grown in more than 20 states. However, 80% of the production is generated in the northwest area (Sonora and Baja California) and the state of Guanajuato in the autumn-winter (A-W) cycle with irrigation; the rest is generated, for the most part, in regions of the central states and the central highlands during the spring-summer (S-S) cycle in rainfed conditions (Peña *et al.*, 2007). During the 2006-2007 to 2010- 2011 period, on average, the nationwide planted area was almost 618 thousand hectares, from which a production of 3.51 million tons was obtained (SIAP, 2012). In the 2007 agricultural cycle case, the states of Sonora, Guanajuato, and Baja California contributed 48, 15, and 15% of the total national production, respectively, (SIAP, 2007).

The national cereal yield average was 5.79 t ha^{-1} during the same period. Considering the average yields per hectare, in 2007 the main producing states were above the national average: Sonora (22%), Baja California (18%), and Guanajuato (7%) (SIAP, 2007). The above denotes that Baja California has favorable environmental conditions for wheat to express a yield potential similar to that obtained in Sonora, but higher compared to the potential yield in Guanajuato states.

Yield potential and protein content of the plant occur when there are adequate soil fertility, climate, water, and other effectively controllable stress problems conditions (Evans, 1993; Kraljevié *et al.*, 1982; Lloveras *et al.*, 2001; Rharrabti *et al.*, 2001; Liu *et al.*, 2003; Guttieri *et al.*, 2005; Peña *et al.*, 2007). However, to maintain the sustainability of this cereal under variable climatic conditions, adequate management of the plants and soil is necessary. In it, adequate nitrogen fertilization doseing has an important influence (Sarkar and Kar, 2008). Nitrogen deficiency in soils decreases grain protein concentration (Fowler *et al.*, 1989; Ottman *et al.*, 2000; Rharrabti *et al.*, 2001). Where protein content is an indicator of soil fertilization or fertility, this deficiency occurs when the plant does not have enough nitrogen in the grain-filling phase (Peña *et al.*, 2007). The grain protein content is an indirect indicator of the amount of protein in the gluten present in semolina and flour, this is important because the gluten content defines the cooking quality of pasta and baking quality (Peña *et al.*, 2007).

Zepeda *et al.* (2007) determined that nitrogen application modified in different proportions the tortilla dough and tortilla quality of different corn used. On the other hand, Guttieri *et al.* (2005) determined that the highest grain yield was obtained with 303 kg of N ha^{-1}; the highest flour and grain protein quality was obtained with 314 kg of N ha^{-1}.

The protein content in grain has great variability (7.1 to 14.7%), from this, it is inferred that the nitrogen fertilization criterion, in quantity or in application times to plants, is not unique, causing the quality of the grain to also vary (Peña *et al.*, 2007). To achieve maximum quality potential expression, plants must be adequately nitrogen supplied (Mengel and Kirkby, 1987).

National and international wheat markets demand this cereal meet quality standards, among which the protein content and percentage of vitreous grain (without white belly) stand out since a high incidence of white belly in the grain is a sign of a low protein content in it (Peña *et al.*, 2007). In this regard, Solís and Díaz (2001) consider that low nitrogen fertilization favors the percentage of white belly in grain. The low protein content is commonly reflected as a white belly problem (Fowler *et al.*, 1989), which in turn causes the industry to pressure farmers on the grain price (Robinson *et al.*, 1979).

To meet the grain wheat quality standards, applying an adequate nitrogen dose for the conditions of the Mexicali Valley, Baja California, is necessary. This research aimed to determine the effect of different nitrogen doses on the yield, grain protein, and percentage of vitreous grain, to recommend the one that most increases grain yield and quality.

MATERIALS AND METHODS

Experiment location

The experiment was conducted in the Experimental Field of the Instituto de Ciencias Agrícolas of the Universidad Autónoma de Baja California, at the Nuevo León ejido, Mexicali, Baja California, Mexico. Located at 32° 24’ 14” north latitude and 115° 12’ 02” longitude west, 12 meters (m) above mean sea level.

Environment at the experimental area

The climate in the area is warm arid, and extreme, with a mean annual temperature of 22.9 °C, 48.5 °C maximum, and −7.0 °C minimum during winter, mean annual precipitation of 60 mm (García, 1988). The soil in the experiment had a clay texture, 4.0 dS m^{-1} electrical conductivity, and a pH of 8.0. Sowing was done in flat terrain with 200 kg ha^{-1} of Aconchi F-76 crystal wheat seed variety.

Vegetative evaluation

The experimental design was of complete randomized blocks with four repetitions in 100 m^2 experimental plots. The treatments were 0, 105, 210, 315, and 340 kg of N ha^{-1} (N_0, N_{105}, N_{210}, N_{315}, and N_{340}, respectively), from urea [$CO(NH_2)_2$], monoammonium phosphate [$(NH_4)H_2PO_4$] and anhydrous ammonia (NH_3). Pre-planting fertilization included 20% of the total applied nitrogen, plus 78 kg of P_2O_5 ha^{-1} in monoammonium phosphate form; in the first and second relief irrigation, 60% more nitrogen was applied, divided into 30% each irrigation, while the remaining 20% was applied during the third relief irrigation.

The harvest took place on June 3, 2010; the grain yield (Yg) was determined in a 2.0 m^2 harvested area based at the center of the experimental plot. The total nitrogen content was assessed following the Kjendhal method, while the protein content was estimated by multiplying the total nitrogen value by 5.7 (Rharrabti *et al.*, 2001). The vitreous grain (without white belly) percentage was obtained from a 100 g grain sample from one seed kilogram processed through the Boerner homogenizer; The grains with white belly were separated from normal ones; The fraction of grains with white belly was weighed and their percentage was determined; the vitreous grain percentage was estimated by subtracting the white belly percentage from one hundred percent.

The data were statistically analyzed using the PROC GLM (SAS Institute, 1996). The comparison of means was determined with the Tukey test ($\alpha \leq 0.05$). The relationship of protein percentage with nitrogen dose and glassy grain percentage was analyzed using the linear regression model.

RESULTS AND DISCUSSION

Nitrogen dosing effect on grain yield

Grain yield occurred with highly significant differences ($P \leq 0.01$). The means comparison indicates that N_0 reported the lowest grain per surface (2.76 Mg ha^{-1}); while the N_{210}, N_{315}, and N_{340} treatments induced similar effects to each other (Figure 1), but the

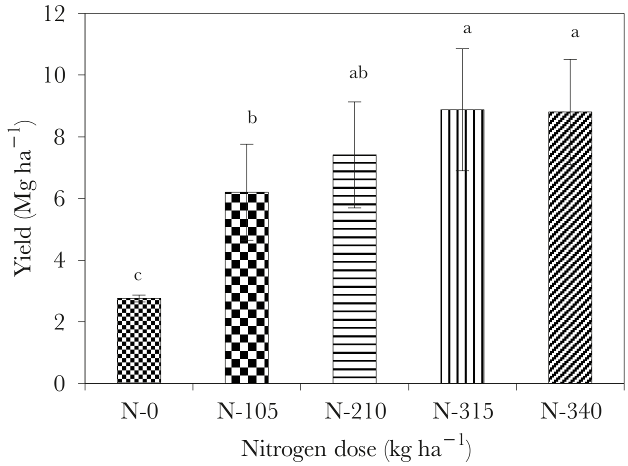

Figure 1. Wheat grain yield relation to nitrogen dosing.

N_{315} and N_{340} treatments increased up to 2.63 Mg ha^{-1} compared to the N_{105} treatment yield, and much higher to that of N0.

Similar results were obtained by Solís and Díaz de León (2001), who reported that as the nitrogen doses increased, from 0 to 120 and from 120 to 240 kg of N ha^{-1} yield also increased, with a maximum of 240 kg of N ha^{-1}. The aforementioned response was also noted (Johnson *et al.*, 1973; Limon *et al.*, 2000; Ottman *et al.*, 2000; López *et al.*, 2002; Guttieri *et al.*, 2005; López *et al.*, 2007; Takahashi *et al.*, 2007, and Ziadi *et al.*, 2008); however, it is also noted that finding an adequate nitrogen dose for wheat cultivation will depend on various factors, such as soil fertility, cultivar, application time, and irrigation and climatic variables. That researchers concluded that the yield response reaches a maximum when applying various nitrogen doses, after which a decrease occurs due to a nutritional imbalance in the plants.

Grain quality (protein)

The grain protein content was highly significant ($P \leq 0.01$) in the N_{210}, N_{315}, and N_{340} treatments compared to the N_0 and N_{105} (Figure 2).

Although no significant differences between the averages among the first three treatments, in absolute values, as more nitrogen was applied, the protein content also increased, such that the N_{340} increased by 5.29, 4.48, 0.67, and 0.39. % in relation to N_0, N_{105}, N_{210} and N_{315}, respectively.

The applied nitrogen doses had a positive linear effect on the protein percentage (Figure 3). This coincides with results by Johnson *et al.* (1973) in three-year research with different nitrogen doses (0 to 135 kg of N ha^{-1}), where the protein content had a positive linear trend to the applied nitrogen doses; likewise, with the results by Fowler *et al.* (1989), Ottman *et al.* (2000), Takahashi *et al.* (2007). However, the same researchers also report that protein content can be affected by various factors related to wheat plants' growth and development since plants in stress conditions due to nitrogen deficiency decreased their grain protein content in response to low nitrogen availability.

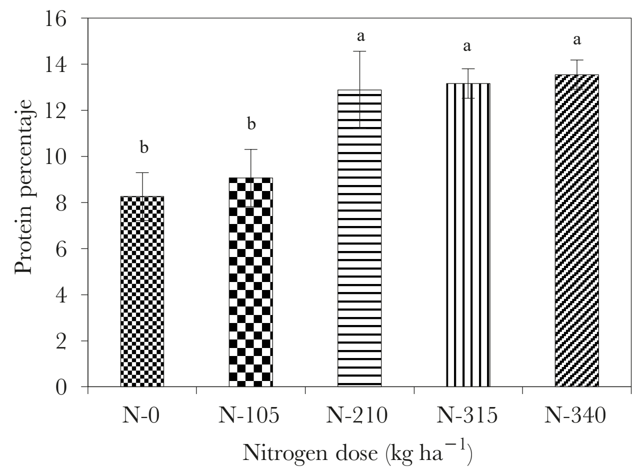

Figure 2. Wheat grain protein percentage relation to nitrogen dose.

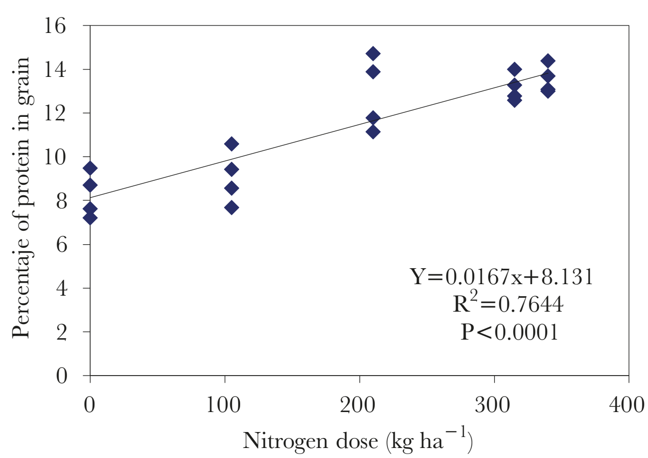

Figure 3. Grain protein content relation to nitrogen dose.

Vitreous grain (without white belly)

The percentage of grains with no white belly had high significant differences ($P \leq 0.01$), so nitrogen dose variation induced grain quality changes; however, the averages of grain without white belly, obtained in the plots fertilized with 210, 315 or 340 kg of N ha^{-1}, were statistically higher than those obtained with 0 or 105 kg N ha^{-1} (Figure 4). In the three highest nitrogen doses cultivated plots, the grain without white belly percentages ranged from 96.93 to 98.78%; still, in the N_0 plot, where wheat was grown with 105 kg N ha^{-1} the percentages were 11.05 and 35.18%, respectively, Thus, according to Peña *et al.* (2007), the increasing percentage of grains without white belly suppose high semolina yields during milling.

The percentage of grains without white belly increased with the nitrogen dose (Figure 5), in such a way that when the percentage of protein increased, the percentage of grains without white belly also increased; That is, as the protein content in the grain decreased,

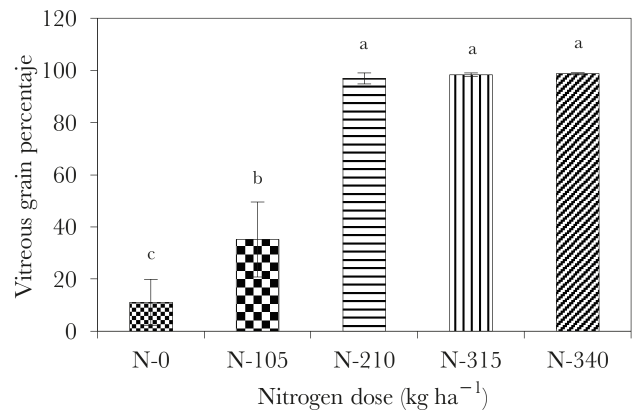

Figure 4. Percentages of grains without white belly relation to nitrogen dose.

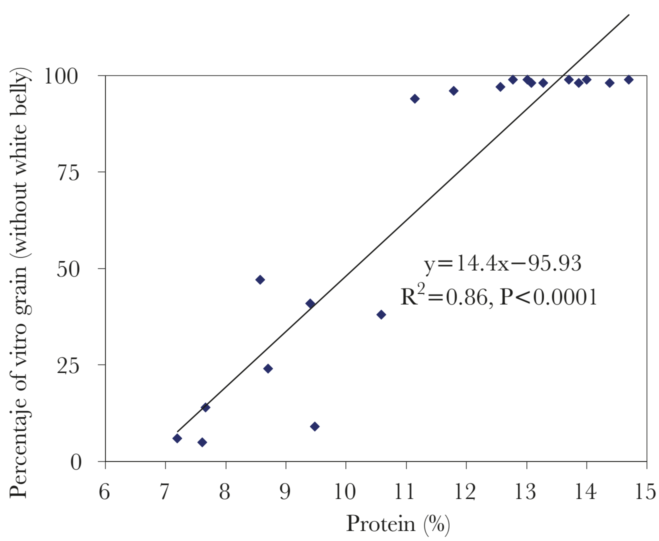

Figure 5. Percentage of vitreous grain (without white belly) relation to wheat grain protein percentage.

the percentage of white belly grains increases. The above coincides with Fowler *et al.* (1989) and Peña *et al.* (2007), since they report that a high incidence of grains with white bellies is a low protein content sign. Likewise, results by Solís *et al.* (2001), reported that the number of irrigations influences the increases in grains with white belly when wheat is fertilized with less than 240 kg of N ha^{-1} since in fertilization with said nitrogen dose, the percentage of grains with white belly decreases to 7%, while in N_0 can obtain up to 70% of white belly grains.

CONCLUSIONS

The 210 to 340 kg of N ha^{-1} doses applied in wheat cultivation allowed greater yield expression, protein content, and grains without white belly so that the grain increased compared to that from grain cultivated with a lower nitrogen amount.

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Colegio de Postgraduados

Effect of aminoethoxyvinylglycine addition on the floral bud opening of *Ranunculus asiaticus* L. cultivars

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ABSTRACT

Objective: To evaluate the effect of aminoethoxyvinylglycine (AVG) additions on several growth variables of *R. asiaticus* in a greenhouse under a typically dry environment of the Mexicali valley.

Design/methodology/approach: The variables determined were, bud opening rate, flower diameter, height of stem and bud growing rate and completely randomized block experimental design under a 2 by 4 factorial arrangement and three replicates was used for setting up the treatments with rates AVG of 0,100,150 and 200 mg L^{-1} over La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) *R. asiaticus* cultivars.

Results: The optimal dose of AVG for reducing flower bud diameter on both cultivars were those of 200 and 100 mg L^{-1} respectively. Also, AVG addition had a significant effect in reducing stem height and growing rate for both cultivars.

Limitations on study/implications: The use of AVG has shown good results as inhibitor of ethylene synthesis and extension of shelf life in carnations, snap dragons (Antirrhinum), ripening of fruits, and to reduce abortion of female flowers in certain species of walnuts, so, based on the above and considering the lack of information regarding the use of AVG inhibitor in the opening of Persian Buttercup flower buds was used AVG in *R. asiaticus*.

Findings/conclusions: AVG addition on plants of *R. asiaticus* had a significant effect reducing growth, both in diameter and height in the La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) varieties.

Keywords: *R. asiaticus*, flower bud growing, stem height, aminoethoxyvinylglycine, diameter ethylene.

INTRODUCTION

In the last decades, the demand for fresh flowers has consistently increased in Europe, EEUU and Japan. The offer comes mainly from countries such as Holland, Italy, Colombia, Kenya, Ethiopia, Turkey, Morocco, China and India. European Union imports account for 60% of world's total production, while EEUU flower imports made up 20%

being Colombia and Ecuador its main suppliers. In 2021 global flower exports reached a total value of 11,000 million dollars. 57.9% of those exports accounted for European union, 24.8% accounted for Latin-American (excluding Mexico but including the Caribbean), Africa participation accounted for 10.4% and finally Asia participation was that of 5.3% (Workman, 2022).

Even though Mexico is not one of the main world flower exporters, since most of its market is local (80%). What is left is exported to EEUU (96.6%) and Canada (3%) (Workman, 2022). In 2021, gross value of Mexico's flower exports overcame 44 million dollars (Exports of cut flowers by country México, 2019). Nowadays, flower industry in Mexico includes an annual production over 96 million dozen of roses, over 12 million dozens of gerbera, over 4 million dozens of sunflower, over 12 million dozens of orchids and almost 187 000 tulips. The State of Mexico concentrates 90% of the country's total production being the only state in Mexico able to export. On the other hand, the state of Baja California exports to EEUU 85% of its flower production (SADER, 2018). One source of economic losses during harvest time is due to the maturation process of the flower which is commonly very fast. Thus, the purchase price is reduced.

Persian Buttercup (*Ranunculus asiaticus* L.) belong to the Ranunculus family of plants. These flowers have been cultivated since the 18th century and have undergone various improvements, both in the number of petals and in the length and consistency of the stems, which has made it possible to increase their availability for marketing as cut flowers, but potentially also for a pot (Scariot *et al.*, 2014). This ornamental plant has the potential to be used more widely in the floral industry, although growers unfortunately face various challenges in growing these plants from their tuberous roots. These challenges include proper root storage conditions, low seed germination, non-uniform growth of crop, and disease susceptibility (Cerveny, 2011).

Crop growth of Persin Buttercup can also be done from seed, although the tuberous roots offer faster and more prolific crops. Tissue culture has also been used for its propagation, although it is an expensive process (Beruto and Debergh, 2004). Life span of cut flowers is highly affected by field production practices, as well as postharvest management, such as the use of preservatives, which may contain biocides and/or ethylene biosynthesis inhibitors to prevent senescence or abscission of leaves and flowers. Since after cutting and before the sale process flowers are exposed to low temperatures to suppress opening and aging process, the size of the bud during cutting has an important impact on its marketing, since the flowers of the Ranunculus family are generally highly sensitive to ethylene additions (Scariot *et al.*, 2009).

There are different products available on the market capable of suppressing or controlling the production of ethylene in different fruits. One of the most effective to ensure that the state of the bud lasts is 1-MCP (1-Methyl cyclopropene), which delays the maturation of the flower and increases its shelf life; unfortunately, this product can only be used in storage conditions, therefore, it is a very highly expensive agricultural supply. A feasible solution is using field ethylene inhibitors to increase the bud stage of the flower during and after cutting (Gamrasni *et al.*, 2017). It has been reported that AGV gives good output when used in the field and sometimes in storage conditions. Ethylene inhibitors act on the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) to reduce flower ripening (Yang and Hoffman, 1984; Schaller and Binder, 2017; Katayose *et al.*, 2021). There is no background information of the use AVG in Persian Buttercup. However, the use of AVG has shown good results as inhibitor of ethylene synthesis and extension of shelf life in carnations (Baker *et al.*, 1977), snap dragons (*Antirrhinum*) (Wang *et al.*, 1977), ripening of fruits (Khan and Ali, 2018) and to reduce abortion of female flowers in certain species of walnuts (Depaepe and Van Der Straeten, 2017). The application of 1mM AGV solution doubled the life cutting span of *lisianthus* flowers, with respect to the control (Shimizu-Yumoto and Ichimura, 2010). AVG doses of 375 mg/L proved to be optimal to reduce the abscission of *Corymbia torelliana* and *Corymbia citriodora* leaves, caused by the generation of ethylene (Trueman and Adkins, 2013). Based on the above and considering the lack of information regarding the use of AVG inhibitor in the opening of Persian Buttercup flower buds, the objective of this study was to evaluate the effect of three doses of AVG on several growth variables of Persian Buttercup such as: bud opening rate, flower diameter, height of stem and bud growing rate over two *R. asiaticus* flower varieties (La Belle Deep Rose (DER) and La Belle Dark Orange (DAR), during the agricultural cycle of 2018-2019.

MATERIALS AND METHODS

Study Site

The experiment was carried out in the autumn-winter 2018-2019 agricultural cycle on land owned by the company Agroproductos y Servicios del Golfo de California SPR de RL, in Colonia Luis Romero Lot 1, Valle de Mexicali, Baja California, Mexico (32° 38’ 22.9” N, 114° 57’ 28” O). The climate in this area is desert type and the summer is characterized by being hot, very dry, with average maximum and minimum temperatures of 49 °C and 16 °C respectively (García, 1988).

Agronomic Practices

Planting was done on 1m by 40 m seed beds with a plant density of 3 bulbs per foot, placed 3 inches deep and 4 inches apart. Prior to sowing, a soil pH analysis was carried out at a depth of 30 cm in three sites of each plot. The results showed an average pH value of 8.2. Before planting, bulbs were treated for 5 min with a 1L ha^{-1} solution of Metalaxil against fungal diseases, following crop guidelines for rose (*Rosa* sp.) cultivation (Álvarez *et al.*, 2013). To measure the diameter of the button, a digital Vernier D-200w brand Caliper with an accuracy of $\pm$0.2 mm was used, while height of the stem, from top to bottom was measured with a Truper® tape measure.

During the growing season, the varieties were attacked by Thrips (*Frankliniella occidentalis*), an insect capable of causing premature wilting, retarding leaf development and distorting shoots, in a very similar fashion to the damage caused in avocado (*Persea americana*) (Castresana *et al.*, 2008). Three applications of Metamidos were made at a dose of 500 mL ha^{-1} in the months of December, January, and February for the control of thrips. Also, three applications of cytokinins (0.204%) 1L ha^{-1} (AGRIMIL plus:Quinival Agroindustrias, Mexico) were added in the months of December and January.

Treatments

A completely randomized block experimental design under a 2 by 4 factorial arrangement and three replicates was used for setting up the treatments. The Combination of the AVG doses of 0 mg L^{-1}, 100 mg L^{-1}, 150 mg L^{-1} and 200 mg L^{-1}, respectively and the varieties La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) formed the different treatments under evaluation.

Fertilization rates of 20-20-20 kg ha^{-1} (NPK) were applied every other day during the first six months of the cycle. On March 4, 2019, the AVG was applied in the flowering stage, when the bud opening reached a diameter between 2 to 3 cm, which was considered the initial measurement. The growth of the bud diameter and stem height were measured every 24 h during a period of three days (0, 0-24 and 0-48 h), which formed the variables: diameter 1, 2 and 3 (DM1, DM2 and DM3), as well as the height of stem 1, 2 and 3 (AT1, AT2 and AT3, respectively). The button diameter growth percentages were analyzed in order to eliminate the effect of different button sizes at the beginning, the growth percentages were analyzed during the first and second day, as well as the overall percentage. During the growth period from 0 to 24 hours (Figure 1), (PD1, PD2), height growth percentage (PA1, PA2) and the total growth percentage for both variables (PDT3 and PAT3). From data of diameter and height of the stem of the plant the percentages of growth in each stage as were calculated as follow:

$$\%\ Growth\ 0-24h = \left(Diameter\ at\ 24h - Initial\ Diameter\right) / \left(Initial\ Diameter\right) \times 100$$
(PD1, PAT1)

$$\%\ Growth\ 24-48h = \left(Diameter\ at\ 48\ h - Diameter\ at\ 24h\right) / \left(Diameter\ at\ 24h\right) \times 100$$
(PD2, PAT2)

$$\%\ Growth\ 0-48h = Diameter\ at\ 48h - Initial\ Diameter) / \left(Initial\ Diameter\right) \times 100$$
(PDT3, PAT3)

Measurements

The following measurements were performed: growth of the bud diameter (DM, cm), stem height (AT, cm), percentage growth of the bud diameter (PD, cm) and percentage growth of the stem height (PAT, %). In a similar fashion, the number of buttons per plant was measured for both varieties in the first cut.

Statistical Analysis

The response variables were analyzed under a 2 by 4 factorial arrangement of treatments under a completely randomized experimental block design. Means treatments were compared using the least significant difference test, also, orthogonal contrasts were performed to determine the trend through VFA doses. The error level used was 5% and the trend was considered the probability between 0.05 and 0.10. The analyzes were performed using the SAS statistical program version 9.4 (SAS Institute, 2020).

RESULTS AND DISCUSSION

Opening of bud diameter

A significant statistical difference (P=0.6) for the interaction AVG × variety was found (Table 1). These results suggest that the presence of aminoethoxyvinylglycine can inhibit the growth process of the uncut flower.

Several studies have found that flowers of other member plants of the Ranunculus family have shown sensitivity to ethylene, including *Aconitum napellus* L., *Anemone × hybrida*, *Delphinium ajacis*, and *Nigella damascena* L. (Cerveny, 2011). Additions with AVG has shown similar results on carnations (Baker *et al.*, 1977) and snapdragon flowers (*Antirrhinum*) (Wang *et al.*, 1977). According to the Yang cycle for the synthesis of ethylene (Yang and Hoffman, 1984), the addition of AVG inhibits the enzyme ACC-synthetase, whose half-life is very short and is found in low concentrations in cells (Li *et al.*, 2020). When comparing the effects of the AVG treatments on the diameter of the flower bud reached at 48 h, it was found that for the DAR variety there were no significant differences between the effects of the doses of 100 and 150 mg L^{-1}, finding the smallest diameter with a reduction of 29% and 33%, respectively, with respect to the control (Table 2, Figure 1); this could be related to a phenomenon observed with the growth of various rose crops (Van Doorn and Kamdee, 2014); where it was found that ethylene inhibited bud opening in various crops, but promoted it in others.

A relatively small dose of ethylene promoted bud opening, while a relatively highly one inhibited it, this is known to be due to differences in the expression of a family of 'DELLA (RhGAI1)' growth repressor genes.

Table 1. Mean values of the effect of the AVG doses on the growth of bud diameter, height, growth percentage, in varieties of *Ranunculus asiaticus* L. during the 2018-2019 cycle.

Variables	mg L^{-1}					Variety			P value		
	0	100	150	200	SE	DER	DAR	SE	Dose	Variety	Dose × Variety
DM1	2.83	3.22	2.61	2.66	0.22	2.72	2.94	0.16	0.246	0.335	0.253
DM2	3.71	3.59	3.11	2.92	0.26	3.27	3.40	0.19	0.145	0.627	0.252
DM3	5.02	4.78	3.85	3.74	0.34	4.20	4.48	0.24	0.039*	0.418	0.060*
PD1	31.65	12.47	18.66	10.85	4.23	20.53	16.29	2.99	0.013*	0.330	0.179
PD2	35.11	33.00	25.96	29.44	9.57	28.55	33.20	6.77	0.910	0.634	0.185
PD3	77.57	50.60	48.00	45.59	13.11	54.71	56.17	9.27	0.312	0.913	0.096
AT1	45.07	51.22	50.58	52.27	1.37	48.57	51	0.97	0.009*	0.096	0.772
AT2	48.48	53.97	52.63	53.75	1.43	51.40	53.01	1.01	0.053*	0.280	0.132
AT3	49.48	55.48	54.18	56.95	1.57	52.30	55.75	1.11	0.022*	0.043*	0.399
PA1	8.12	5.62	3.93	2.87	3.01	6.25	4.02	2.13	0.636	0.469	0.370
PA2	2.15	2.74	3.06	5.93	1.62	1.72	5.22	1.14	0.384	0.046*	0.115
PA3	10.32	8.45	7.05	8.93	3.22	8.03	9.35	2.27	0.9116	0.6875	0.4105

DM1:DM2:DM3=Diameter 1, 2 y 3, respectively. DER=Deep Rose variety, DAR= Dark Orange variety. PDB1:PDB2:PDB3=Bud diameter growth percentage 1, 2, 3 respectively. PAT1:PAT2:PAT3=Stem height growth percentage 1, 2 y 3, respectively. SE=Standard error. AVG=aminoetoxiviniglicina; * P≤0.05.

Table 2. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter after 48 h during the 2018-2019 agricultural cycle.

Variety	AVG Dose (mg L^{-1})	Flower bud diameter Mean value	Statistical
Deep Rose	0	4.17	a
	100	5.37	b
	150	3.8	a
	200	3.47	a
Dark Orange	0	5.87	b
	100	4.18	a
	150	3.89	ab
	200	4.01	ab

Means with the same letter are not statistically different ($P \leq 0.05$).

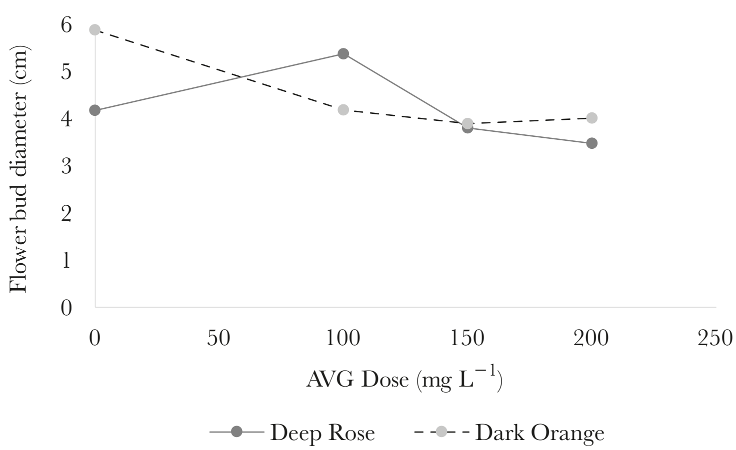

Figure 1. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter after 48 h during the 2018-2019 agricultural cycle.

That is, due to the differences in the amount of ethylene generated by various *R. asiaticus* cultures (Scariot *et al*, 2009), the same doses used in this study can generate different degrees of inhibition of bud growth. The differences in sensitivity, as well as in the promoter-inhibitor role of bud growth, have also been related to the variation between crops in the concentrations of other phytohormones, mainly cytokinins, gibberellins, abscisic acid (ABA) and auxins (Iqbal *et al.*, 2017).

For the DER variety, the smallest diameter of the bud was found at a concentration of 200 mg L^{-1} (17%) lower than the control ($p<0.05$) (Table 2 and Figure 1). The addition of aminoethoxyvinylglycine showed a positive effect for both varieties from the lowest dose, although for the DAR variety there was no further decrease with higher doses, which did occur with the Deep Rose variety; such a difference was observed at twice the dose, that is, at 200 mg L^{-1} (Figure 1).

Finally, Figure 2 shows the global average outputs for both growing periods, it was confirmed that the lowest growth percentage of *R. asiaticus* buds was achieved at an aminoethoxyvinylglycine concentration of 100 mg L^{-1} (24%) for the DAR variety, while

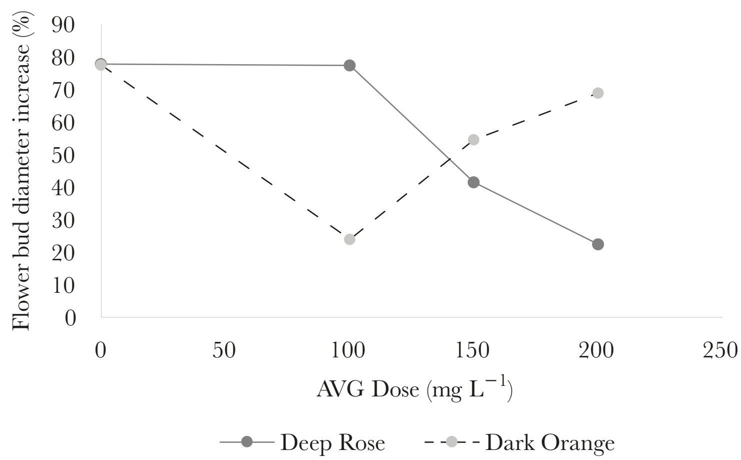

Figure 2. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter increase after 48 h during the 2018-2019 agricultural cycle.

for the DER variety, the lowest percentage of growth is obtained with 200 mg L^{-1} (22%). In some cases, even when the differences do not reach the level of significance established in the analysis; there are important results from the point of view of quality control during the harvest, since the reduction in the speed of the opening of the button allows to reduce the percentage of buttons that exceed the diameter specifications established by the customers.

In addition to the differences in the sensitivity of different *R. asiaticus* cultivars to ethylene generated from methionine, it is necessary to evaluate the effect of exogenous ethylene (Scariot *et al*, 2009). The reduction in the diameter of the bud could be related to environmental ethylene coming from the cutting of contiguous *R. asiaticus* buds, however, it is necessary to carry out tests under isolation conditions to evaluate this hypothesis. Some researchers have found that for *R. asiaticus* cut flowers, the addition of ACC synthetase inhibitors, such as aminooxyacetic acid (AOA) and sodium thiosulfate (STS) at a maximum concentration of 48 mg L^{-1}, had no effect in their senescence (Kenza *et al.*, 2000), (Belding and Lokaj, 2002). Other authors found that the use of STS and AOA in concentrations of 300 to 350 mg L^{-1} increased the post-harvest life of 2 and 4 different types of *R. asiaticus*, respectively; although they also found two types of cultivars of the same species that were insensitive to ethylene (Scariot *et al.*, 2009).

The results obtained in this study showed that the addition of AVG in doses of 100 to 200 mg L^{-1} does have an effect on the reduction of ethylene generated during the bud opening process in live flowers, also, considering that ethylene is a multifunctional phytohormone, in the sense that is capable of regulating growth and senescence processes (Iqbal *et al.*, 2017). In another study, it has been reported that by applying AVG to pears during a two weeks period at early ripening and cold temperature, high fruit firmness was obtained due to the reduction in respiration rate and ethylene production (Khan and Ali, 2018).

On the other hand, in a study conducted by Doerflinger *et al.* (2019) AVG and 1-MCP were used to evaluate their effect on delaying apple ripening and therefore reducing fruit dropping, thus, facilitating harvest. It was found that AVG acted as an intermediate in the internal production of ethylene, between week 1 and 4, while it did not affect neither softening nor the rate of loss of ethylene concentration starch during the storage period. Moreover, the use of AVG and 1-MCP before harvest affected the reliability of the starch concentration since a harvest index cannot be satisfactorily met. The effects of both inhibitors on starch hydrolysis depend on factors such as cultivar and growing season (Doerflinger *et al.*, 2019).

Plant Height

As far as plant height, either AVG doses and variety were statistically significant for AT1 (P=0.009), AT2 (P=0.053), AT3 (P=0.022), and in AT3 both effects (P=0.022 and P=0.043) respectively (Table 1).

The effect of AVG treatments at 48 h showed that for the Dark Orange and Deep Rose varieties, the minimum percentage of growth was at a dose of 100 mg L^{-1} (7%) and 150 mg L^{-1} (6%), with respect to the control ($p<0.05$) (Table 3). Accordingly, an increase in the AVG dose would imply a greater growth as expressed in the height of the plant compared to the control. However, it has been found that the development of leaves and flower stems were affected by the presence of ethylene and other phytohormones, such as auxins, gibberellins and cytokinins (Iqbal *et al.*, 2017). Several studies have shown that in *Arabidopsis* plants treated with AVG, ethylene promoted cell division, increasing stem mass, except for plants exposed to environmental stress, where ethylene had a negative effect on the cell cycle (Barry and Giovannoni, 2007; Dubois *et al.*, 2018).

Furthermore, despite the fact that height percentage increase of the stem was not significantly affected by the treatments ($P>0.05$); the observed changes for each dose were similar to those found in the button diameter percentage increase. In other words, the growth regulation processes mediated by ethylene for the crops examined in this study were similar both in the stem and the flower bud. Apparently, the analysis carried out on growth percentages showed similarities in the growth patterns of both parts of the flower, as opposed to the analysis of the absolute value of the diameter of the flower bud or the length of the stem (Figure 3).

Table 3. Mean values of the effect of AVG doses upon plant height after 48 hours during the 2018-2019 agricultural cycle.

Variety	AVG Dose (mg L^{-1})	Plant height Mean value (cm)	Statistical
Deep Rose	0	47.84	a
	100	55.88	a
	150	50.51	a
	200	54.61	b
Dark Orange	0	51.11	a
	100	55.02	a
	150	57.57	b
	200	59.26	ab

1) means with the same letter are not statistically different ($P\leq0.05$).

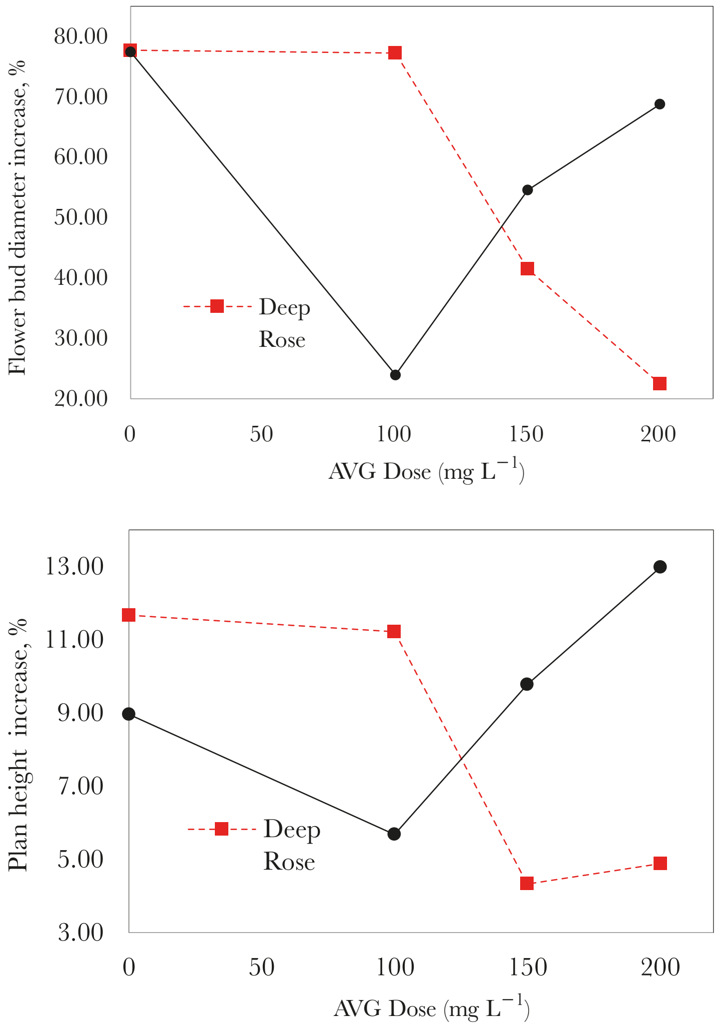

Figure 3. Mean values of the effect of AVG doses upon plant height and flower bud diameter after 48 hours during the 2018-2019 agricultural cycle.

CONCLUSIONS

AVG addition on plants of *R. asiaticus* had a significant effect reducing growth, both in diameter and height in the La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) varieties. The optimal AVG dose to reduce bud diameter growth for the Deep Rose variety was 200 mg L^{-1}, while for the Dark Orange variety was 100 mg L^{-1}. In addition, plant height of both varieties increased as AVG doses increased. Reduction in the diameter of the flower bud could be related to environmental ethylene from the cutting of contiguous *R. asiaticus* flower buttons, in any event, it is necessary to carry out further tests under isolation conditions.

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Colegio de Postgraduados

Silicon fertilization effects in *Pinus devoniana* Lindl. in nursery stage

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ABSTRACT
Objective: To evaluate the effect of applying silicon products on morphological variables, quality indices, and mineral content of *Pinus devoniana* seedlings during the nursery stage.
Design/Methodology/Approach: Four-month-old nursery seedlings produced on substrate were used. These were supplied with silicon in soluble powder applied on the substrate, and foliar liquid presentation and then evaluated in their morphological variables, foliar mineral content, and quality indices.
Results: The application of soluble silicon powder had a positive effect on stem length; while the stem diameter was favored by both applications, which improves its storage capacity. Regarding plant biomass production, the application of soluble silicon powder resulted in higher values of aerial biomass, while root production was favored by foliar liquid application. Plant quality was not affected by the silicon application from either the soluble powder or the liquid; however, the liquid foliar application had the best effect for determining variables of the species. The silicon application did not affect other essential element absorption.
Limitations/Implications of the study: The results and conclusions are limited to *Pinus devoniana* plants in their nursery stage under the described management and substrate conditions.
Findings/Conclusions: The silicon application favored growth, and did not affect the plant's quality or other elements' absorption. The soluble powder was positive for stem length, and the foliar application for root development benefited the stem diameter.

Keywords: Preconditioning, plant quality, fertilization, *Pinus devoniana*.

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INTRODUCTION

In Mexico, conservation programs for the *Pinus* genus are not efficient (Sánchez *et al.*, 2008), which is why reforestation is the best option (Sáenz, 2004). In this sense, *Pinus devoniana* Lindl. stands out for its economic and ecological importance, as well as its wide usage in reforestation programs (Perry *et al.*, 2000). In addition to the above, the

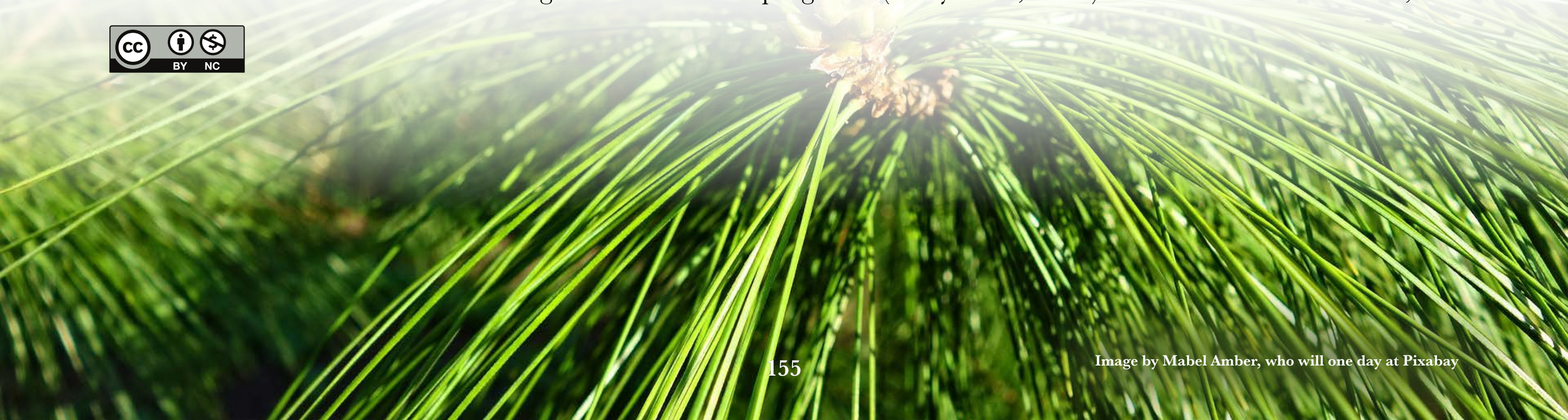

Image by Mabel Amber, who will one day at Pixabay preconditioning stage in the nursery is essential for these plants, because the best quality ones are selected for their morphological and physiological characteristics (Ramírez and Rodríguez, 2004), which will depend on their genetic characteristics and the techniques implemented in the nursery (Prieto *et al.*, 2009).

In this sense, the main evaluated morphological attributes were the stem height, neck diameter, height-diameter relationship, biomass, and leaf and root area (Escobar and Rodríguez, 2019); among the physiological attributes, the macronutrients concentration (nitrogen, phosphorus, potassium, among others) and micronutrients (iron, manganese, zinc, among others) were evaluated (Quiroz *et al.*, 2009).

Therefore, silicon is among the mineral elements that can help produce plants with adequate morphological and physiological quality; a beneficial element, Si contribution is expected to improve plant quality (Ma *et al.*, 2001). However, its optimal requirement is not yet well defined, nor is its physiological effect on different quantities (Tubana *et al.*, 2016). In the active form, absorption is reported in the 2 to 9 pH range (Epstein, 1994) by the roots in a monosilicic acid $Si(OH)_4$ solution (Loué, 1988).

In agriculture, this element can stimulate growth (Loaiza, 2003), and productivity, since it contributes to P, Ca, Mg, K, and B availability (Epstein and Bloom, 2005). For this reason, it is necessary to develop technologies and implementation of silicon-based fertilizers, which help plants during their first years of life after their planting in the field. The objective of this research was to evaluate the effect of silicon-based product applications on the morphological, physiological variables, and quality indices of *Pinus devoniana* Lindl plants during their nursery stage.

MATERIALS AND METHODS

Study site

The experiment took place at Valle de Ameca S. P. R. de R.L. Forest Nursery, located at 20° 33' N and 104° 3' W, 1235 m altitude, in Ameca, Jalisco state, Mexico. The local climate is semi-warm, subhumid with summer rain, medium humidity, temperatures ranging from 16-24 °C, and 800 to 1100 mm annual precipitation (INEGI, 1999).

Plant production for evaluation

For the experimental work, four-month-old *Pinus devoniana* Lind seedlings were evaluated. Their initial morphological characteristics and mineral content are shown in Table 1.

Table 1. Morphological characteristics and leaf mineral content of assessed *Pinus devoniana* seedlings.

Morphological characteristics					
Height (cm)	**Diameter (mm)**	**Air weight (g)**	**Air vol. Aéreo (cm^3)**	**Root weight (g)**	**Root vol. Raíz (cm^3)**
4,8±1,3	2,8±0,05	0,6±0,03	2,9±0,04	0,4±0,14	13,2±0,29
Leaf mineral content					
N (%)	**P (%)**	**K (%)**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**
1,8±0,07	0,24±0,01	0,84±0,02	416,3±0,17	427±0,27	69±0,49

Vol.: volume; N: nitrogen; P: phosphorus; K: potassium; Mn: manganese; Fe: iron; Zn: zinc.

The seedlings were produced in 60-cavity polystyrene trays, 160 cm^3 per cavity, at Valle de Ameca forest nursery. The substrate for germination and plant production was a mixture of 50% peat moss type peat, 49% pine bark (less than 5 mm), and 1% agrolite; with 84% total porosity, 22% aeration porosity, 62% water retention capacity, 27 mm particle size (weighted average diameter) and 0.26 g cm^{-3} apparent density.

The production conditions followed nursery protocols. The plants were kept under 50% shade mesh and fertilized with 7-40-17 Multicote™ (N-P-K) fertilizer formula, applied throughout the initial growth phase (from 15 to 120 days after germination). After 120 days, only silicon and continuous irrigation were applied to all plants.

The plants' morphological characteristics and foliar mineral content at the beginning of the experiment, shown in Table 1, were assessed 120 days after germination.

Experiment establishment and treatment application

For the experimental silicon fertilization (SiO_2), the Silik-Tek® commercial fertilizer was applied in two presentations, soluble powder and liquid. These were diluted in different concentrations according to the treatments. The liquid form was foliar applied, while the powder presentation was applied diluted directly to the substrate. Five treatments, described in Table 2, were considered.

The treatments were distributed in a 2^2 factorial design; with random sampling, consisting of 4 repetitions with 30 experimental units per treatment. The control was not supplied with silicon. In the treatments, 6 applications were done, one every 15 days, for three months (February-April). The plants were kept in growth beds under 50% netting; irrigation was daily applied in a uniform and localized manner.

For both presentations, the dosage was done following the commercial product recommendations. Furthermore, considering that higher plants contain between 0.1 and 10% silicon based on their dry weight, a lower dose of 100 mg/L and 0.1 mL/L and a higher dose of 300 mg/L and 0.3 mL/L were used to have a better evaluation range.

Growth variables, quality indices, and mineral content

After six months, including the 90 days of treatment application (February-April), in May, the morphological evaluation was done through destructive sampling. 30 plants were randomly selected per treatment, removed from their trays, the substrate containing their roots also removed and their aerial structures separated from the root. Their length (cm)

Table 2. Treatment distribution for silicon application (SiO_2).

Treatment	Silicon business presentation (SiO_2)	Silicon dosage
T0	Witness (No silicon)	0
T1	Solid (Via substrate)	100 mg/L
T2	Solid (Via substrate)	300 mg/L
T3	Liquid (Via foliar)	0.1 mL/L
T4	Liquid (Via foliar)	0.3 mL/L

Note: The control (T0) refers to the plants that were maintained under the same management conditions in the nursery, without silicon fertilization.

and stem diameter (mm) were measured. The length was evaluated with a millimeter ruler from the neck to the apical bud. The diameter was assessed at the base of the stem (above the root collar) with a digital vernier. Aerial and root biomass was determined in dry weight (g), for which the aerial parts and root were separated, the evaluated plants were extracted from their tray and immersed in water to remove excess substrate; they were subsequently placed in paper bags and placed in a 70 °C oven for 72 h until constant weight. Finally, they were separately weighed on a Sartorius® analytical balance with a 1 mg precision.

With the morphological variables, the following quality indices were determined:

a) Robustness index (RI): evaluates the relation between the height (cm) and the diameter at the root neck (mm) (Prieto *et al.*, 2009):

$$RI = \frac{Height\,(mm)}{Root\ neck\ diameter\,(mm)}$$

b) Ratio: aerial dry weight/root dry weight (Thompson, 1985):

$$R\,RDW\,/\,ADW = \frac{Root\ dry\ weight\,(g)}{Aerial\ dry\ weight\,(g)}$$

c) Dickson quality index (DQI): evaluates the morphological plant characteristics; the higher the index values, the better the plant quality (Dickson *et al.*, 1960):

$$DQI = \frac{Total\ dry\ weight\,(g)}{\frac{Height\,(mm)}{Diameter\,(mm)} + \frac{Dry\ weight\ of\ aerial\ part\,(g)}{Dry\ weight\ of\ root\,(g)}}$$

d) lignification index (Prieto *et al.*, 2004b):

$$LI = \left(\frac{Total\ dry\ weight\,(g)}{Total\ fresh\ weight\,(g)}\right)100$$

The foliar concentration of essential macro and micronutrient elements was quantified in dry plant needles, each sample consisted of 150 needles (experimental unit). Leaf analyses were conducted using different techniques and equipment; Nitrogen was determined following the micro-kjeldahl method. Phosphorus and potassium were assessed via wet digestion in a (LAMBDA 850) spectrophotometer; the microelements were determined by atomic absorption, in a 240F Varian equipment.

Statistical analysis

A normality test was performed (Chi-square and Shapiro-Wilk W statistic). Subsequently, the data were subjected to an analysis of variance (ANOVA) on all the evaluated growth and leaf mineral content variables using the Statgraphics Centurión XVII (Statgraphics, 2014) statistical software.

RESULTS AND DISCUSSION

Growth variables

The analysis of variance showed significant differences between treatments ($p \leq 0.05$), which indicates that *Pinus devoniana* plants in the nursery stage pretreated with silicon product, have a different response according to the silicon product presentation, form of application, and dose delivered, evidenced by the results reported here regarding the evaluated morphological variables (Table 3).

In this sense, it is important to mention that species with tussock-type growth such as *Pinus devoniana*, *Pinus montezumae* Lamb., and *Pinus engelmannii* Carr. (Calderón, 2006; Prieto *et al.*, 2018), they first develop their storage capacity during their first years (stem diameter and root volume) rather than height growth (stem length), already been described in other works (Ávila *et al.*, 2014; Rosales *et al.*, 2015). This is because there is little elongation of the epicotyl, greater needle production and elongation, as well as a greater increase in stem diameter, which was also recorded in this research. Despite this, important results were found that will help make decisions in nursery management and plant preparation of species with this growth type, so that they achieve higher plant quality before transplant to the field.

Regarding the stem length and its relation to the control treatment (T0), the soluble silicon powder applied to the substrate (T1 and T2) were the ones with the highest values (8.1 and 8.2 cm, respectively), followed by the high-dose foliar application (T4, reaching 7.8 cm); while the foliar low-dose treatment (T3) showed the lowest value for this variable (6.1 cm) (Table 3). Also, regarding stem diameter, silicon treatments showed positive results regardless of the dose, presentation, or form of application (T1, T2, T3, and T4), with a significant statistical difference compared to the control (T0) which had the lowest value (5.2 mm). This means that the silicon application favors the storage capacity that will be essential for its subsequent adaptation to field conditions. In addition, a larger stem

Table 3. Stem length and diameter, aerial and root biomass production.

Tratamiento	Length (cm)	Diameter (mm)	Biomass (g)	
			Aerial	Root
T0	$7{,}8\pm0{,}76^{b}$	$5{,}2\pm0{,}02^{c}$	$1{,}9\pm0{,}01^{c}$	$1{,}3\pm0{,}01^{c}$
T1	$8{,}1\pm0{,}70^{ab}$	$6{,}3\pm0{,}07^{a}$	$3{,}5\pm0{,}13^{a}$	$1{,}4\pm0{,}03^{bc}$
T2	$8{,}2\pm0{,}13^{a}$	$6{,}3\pm0{,}07^{a}$	$2{,}3\pm0{,}04^{b}$	$0{,}9\pm0{,}02^{d}$
T3	$6{,}1\pm0{,}78^{d}$	$6{,}2\pm0{,}04^{b}$	$1{,}5\pm0{,}03^{d}$	$1{,}5\pm0{,}03^{b}$
T4	$7{,}4\pm0{,}86^{c}$	$6{,}4\pm0{,}06^{a}$	$1{,}7\pm0{,}07^{cd}$	$2{,}6\pm0{,}09^{a}$

Different letters in the same column indicate significant differences. *No significant differences were found, according to the Tukey ($P<0{,}05$).

diameter has been related to better vigor and higher survival. In this matter, similar plants with diameters greater than 5.0 (mm) show greater bending and pest resistance, and better plant quality (Mexal and Landis, 1990; NMX-AA-170-SCFI-2016; Prieto *et al.*, 2009). In different species, it has been shown that a larger root neck diameter in tussock-type growth species favors the adaptation of plants to the planting site (Tsakaldimi *et al.*, 2013).

Regarding the aerial biomass accumulation, significant differences ($p \leq 0.05$) were observed between the treatments as shown in Table 3, finding that the highest averages were presented in the treatments with diluted silicon powder to the substrate (T1 and T2), with values of 3.4 and 2.3 g, respectively. The foliar application treatments showed no positive effect on the aerial structures (T3 and T4), the control treatment (T0) showed a better effect than the latter (1.9 g). The above demonstrates that the generated aerial biomass is related to the water storage capacity that occurs with growth since the stem functions not only as support and conduction (Casas, 2001) but as a storage organ. In this sense, larger stem diameter and aerial biomass were recorded due to the contribution of the plant needles in the treatments with substrate applications (T1 and T2). This shows that the roots had greater silicon solution absorption and translocation to the aerial structures via the substrate than the silicon absorption via foliar, possibly due to the waxy epidermis physical barrier of the plant needles that limited foliar solution absorption.

On the other hand, regarding root biomass production, there were significant differences in the application route and the dose, where the foliar application treatments of silicon at both doses (T3 and T4) reported the highest values (1.5 and 2.6 g, respectively). This shows that the silicon absorbed from the foliar application mobilizes to the plant's root along with the photosynthates. The treatments applied to the substrate (T1 and T2) did not favor root development like the control (T0), reflected in its lower biomass, even in high dose (T2) substantially affecting its growth (0 .9 g). Based on the above, García *et al.* (2015), when evaluating *Pinus engelmannii* Carr. growth under various environmental conditions and fertilization during the preconditioning stage, found that the root biomass was greater in outdoor and outdoors plus fertilization conditions, resulting in 1.3 and 1.2 g, respectively. This is corroborated by our results, where fertilization with both doses generated a greater total biomass. Berendse *et al.* (2007) and Camargo and Rodríguez (2006) mention that plants under low fertilization regime allocate greater biomass to the roots to promote greater growth.

Quality indices

All treatments presented significant differences ($p \leq 0.05$), which is reflected in the reported values (Table 4).

In general, silicon applications did not affect the quality of the plants; however, the foliar treatments (T3 and T4) presented the highest values for the evaluated quality indices (Table 4).

The ratio index between root dry weight and aerial dry weight (RDW/ADW) evidenced the biomass production relation which reflects the development of the plant in the nursery (Sáenz *et al.*, 2014). In this sense, an equal-to-one ratio indicates that the aerial weight is equal to the root weight. However, if the value is less than one, it implies that the root weight

Table 4. Quality indices in *Pinus devoniana* Lindl.

Tratamiento	R: PSA/PSR	ICD	IR	IL
T0	1,5±0,01[b]	0,19±0,01[c]	1.6±0,14[a]	19±0,11[d]
T1	2,5±0,04[a]	0,18±0,00[c]	1.3±0,05[b]	23±0,29[c]
T2	2,6±0,02[a]	0,26±0,00[b]	1.1±0,05[d]	25±0,15[b]
T3	1,0±0,00[c]	0,36±0,01[a]	1.0±0,07[e]	27±0,30[a]
T4	0,7±0,01[d]	0,35±0,00[a]	1.2±0,04[c]	25±0,08[b]

Different letters in the same column indicate significant differences. *No significant differences were found, according to the Tukey ($P<0,05$). R: PSA/PSR (ratio: air dry weight / root dry weight), ICD (quality index Dickson), IR (Robustness index), IL (lignification index).

is greater than the aerial dry weight; on the contrary, greater than one value indicates that the aerial dry weight is greater than the root weight (Rodríguez, 2008).

The treatment that reported the best plant ratio was treatment T3 (1.0), while treatments T2 and T0 had greater than one value, indicating that their plants would have lower underground biomass production (2.6 and 2.5, respectively). Rueda *et al.* (2012), evaluating *P. devoniana* plant quality produced in nurseries in Jalisco state, presented values of 7.5 to 2.0, respectively, which indicates it is a robust species and bending resistant. Likewise, Rosales *et al.* (2015) and García *et al.* (2015) in *P. engelmannii* Carriére found reported values of 3.2 to 6.1; however, Rueda *et al.* (2014) recommend a <2.0 value for this index.

The Dickson index evaluates the best morphological parameters, since it shows the balance between the distribution of biomass and robustness, indicating that the higher the index, the better the plant quality (Birchler *et al.*, 1998). In this sense, significant differences were observed between the treatments, finding that the treatments that presented the highest values were T3 and T4 with 0.36 and 0.35, respectively, while the lowest values were obtained in the treatments T0 and T1 with 0.19 and 0.18, respectively; the results agree with what was found by Bautista *et al.* (2018), where *Pinus greggii* Engelm., with controlled delivery fertilization presented values of 0.26 to 0.22. In agreement, with Reyes *et al.* (2005), high values indicate good balance and development of the plant, which evaluates different combinations of morphological parameters (Dickson *et al.*, 1960).

The robustness index relates the plant height and the root neck diameter, being an indicator of the plant's resistance to wind relating to their survival. Its value should be less than 6, since a lower value indicates better quality plants with shorter and more robust trees, while greater than 6 values show growth in height and diameter inequality, generating long but thin stems (Prieto *et al.*, 2009). In addition to the above, significant differences were present, the highest values were reported in treatment T0 followed by T1 with (1.6 and 1.3, respectively), while the lowest values were reported in T4, T2, and T3. with (1.2, 1.1, and 1.0, respectively). These concur with results reported by Sáenz *et al.* (2014) evaluating *Pinus devonia* Lindl plant quality with 1.2 values. This relation between plant height and diameter indicates that the lower its value, the shorter and thicker it will be, which is favorable for environments with humidity limitations (Rodríguez, 2008).

The lignification index assesses the percentage of dry weight in relation to the water supply in the plants, indicating their pre-conditioning level, since values between 25 and

30% represent optimal lignin values in conifers (Prieto *et al.*, 2009). Therefore, significant differences were found, where the optimal value was observed in treatments T2, T3, and T4 (25, 27 and 25%, respectively). In addition to the above, the found values provide an estimate of the robustness degree a plant needs to tolerate water stress. At the plantation site, the only treatment with foliar fertilization and a high dose is the one within the reported values. However, the two low-dose treatments with two fertilizations were close to that mentioned above. Prieto *et al.* (2004a) and Ávila *et al.* (2014), when evaluating the moisture availability reduction as preconditioning, *P. engelmannii* had values of 29.2, 22.9 and 24.3%, which is related to the results obtained here.

Leaf mineral content

In the macronutrients and micronutrients foliar mineral content, as shown in Table 5, significant statistical differences were found, except for the phosphorus content. In this sense, fertilization in the nursery is of utmost importance since it affects one of the most important critical components in developing high-quality plants in a nursery (Landis and Dumroese, 2009).

In the nitrogen content, significant differences were observed between treatments, the highest accumulation percentage occurred in T3 at 1.8%, followed by treatments T0, T1, and T2 with 1.7, 1.5, and 1.4% values respectively. While the lowest accumulation was in T4 with 1.2%. According to Landis (1985), the N range in this treatment was slightly below the established values. Gutierrez *et al.* (2015), when evaluating the pH of irrigation water (8 and 5.5) and fertilization (50-123-73 of N, P, K), in *Pinus cembroides* Zucc., the four evaluated treatment values of 1.6 to 1, 5, found that these coincide with that was reported here, where fertilization with silicon in both doses did not affect this element absorption.

There were no significant differences in the phosphorus content, this mineral ranged from 0.29 to 0.27% between treatments. Compared to that reported by Landis (1985), all treatments had minimum concentrations. In this sense, fertilization and both doses did not affect this mineral concentration in the plants.

In the potassium concentration, significant differences were present, where the highest value was found in T3 with 0.88%, followed by treatments T1, T2, and T4 with

Table 5. Foliage nutrient content in *Pinus devoniana* Lindl.

Tratamiento	N	P *	K	Mn	Fe	Zn
	%			mg kg^{-1}		
T0	1,7±0,21[ab]	0,28±0,01	0,82±0,01[b]	299±14[ab]	99±2,3[b]	31±0,61[a]
T1	1,5±0,12[ab]	0,28±0,00	0,84±0,02[ab]	308±6,5[ab]	129±7,2[a]	26±0,63[ab]
T2	1,4±0,07[ab]	0,27±0,01	0,83±0,02[ab]	293±8,5[b]	103±1,4[b]	23±0,34[b]
T3	1,8±0,30[a]	0,29±0,01	0,88±0,01[a]	316±12[ab]	113±3,2[ab]	27±0,88[ab]
T4	1,2±0,05[b]	0,28±0,01	0,83±0,02[ab]	327±6,3[a]	111±7,2[b]	30±3,00[a]
Rango (Landis, 1985)	1,3 a 3,5	0,20 a 0,60	0,70 a 2,5	100 a 250	40 a 200	30 a 150

Different letters in the same column indicate significant differences. *No significant differences were found, according to the test Tukey (P<0,05).

concentrations of 0.84, 0.83, and 0.83, respectively, the lowest value was for treatment T0 with 0.82%.

These results are within the ranges recommended by Landis (1989), for this mineral, slightly above the minimum value. In addition to the previous, our results agree with what is established for grass-growing species. Muñoz *et al.* (2015), when evaluating plant quality in a nursery at Zitácuaro municipality, Michoacán state, found that *P. devoniana* had a 0.68% value, considered of low quality. Based on the obtained results, Escobar and Rodríguez (2019) propose a starting point to refine and reduce nutritional variables, given the great diversity of Mexican forest species.

There were significant differences between treatments in the evaluated contents of micronutrients. Manganese concentrations had significant differences, T4 with 327 mg kg^{-1} was the treatment with the highest value, followed by T3, T0, and T1 (316, 299, and 308 mg kg^{-1}, respectively), the lowest value occurred in T2 with 293 mg kg^{-1}. Landis (1985) mentions that the recommended concentrations for this micronutrient range from 100 to 280 ppm. The results in all treatments exceed the optimal values, which might indicate a greater accumulation of this element with silicon fertilization in both doses, as well as in the control treatment, since the plants use micronutrients at low concentrations, as is the case of potassium, which functions as an organic structure constituent (Toro and Quiroz, 2007).

Significant differences were found in the iron content, where the highest concentration occurred in treatment T1 with 129 mg kg^{-1}, followed by T3 with 113 mg kg^{-1}, while the lowest occurred in T4, T2 and then the control (111, 103, and 99 mg kg^{-1}). Those results obtained here are within the concentrations recommended by Landis (1985), which range from 40 to 200 ppm. When evaluating plants falling in a *P. devoniana* nursery Bernaola *et al.* (2015) found that the iron content in 1 and 5 L containers with fertilization had 299 and 94 ppm, respectively, while without fertilization in 1 L and 5 L containers, the values ranged from 123 to 126 ppm.

Zn concentrations reported significant differences between treatments, T0 and T4 had the highest values with (31 and 30 mg kg^{-1}, respectively), followed by T3 and T1 with 27 and 26 mg kg^{-1}, each. While the lowest value occurred in T2 with 23 mg kg^{-1}. According to Landis (1985), only T0 and T4 with high foliar fertilization were within the minimum optimal, while the other treatments reported low absorption of this microelement. According to Foucard (1997), this micronutrient insufficiency generates mottled chlorosis in young leaves, followed by necrosis and leaf fall.

CONCLUSIONS

Supplying *Pinus devoniana* seedlings silicon when produced in trays and a forest substrate mixture during their nursery preconditioning stage favors their growth, does not affect their quality or other essential elements absorption, and its application will depend on the expected effect.

Applying a soluble silicon powder product directly to the substrate favors the length, stem diameter, and aerial biomass. The foliar application of silicon favored the stem diameter and root biomass. The low soluble powder dose to the substrate and the high foliar liquid dose applied showed the best growth results for the species in the benefited variables.

The quality indices revealed that the foliar liquid silicon application showed the highest Dickson index values; while the shoot/root ratio (ADW/RDW) was the application of soluble powder to the substrate and the treatment without silicon. The robustness was higher in the control, while for the lignification index, all silicon treatments were higher.

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